Quantitative genetic aspects of breeding for resistance to gastrointestinal parasites in small ruminants

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Thesis presented for the degree of Doctor of Philosophy

University of Edinburgh

2002

Acknowledgements

First of all I would like to acknowledge Prof. S.C. Bishop for all the help he has given me during my time in Roslin Institute. He has been a really excellent supervisor. Thanks to my second supervisor Prof. W.G Hill for his part in this thesis. The chapter of the random regressions would have been impossible to complete without the help of I. White.

I would like also to thank all the staff and students of the Genetics and Biometry Division of Roslin Institute. Special thanks to Dr. Ricardo Pong-Wong, Dr. Katrin Mackenzie, Jules Hernandez, Pau Navarro, Heli Wahlroos and Victor Martinez for their friendship and help with various matters all these years.

It would have been impossible to acknowledge all the relatives and friends who have been supporting me through out these years. The 'Greek company' have been also excellent friends and have made my staying in Edinburgh unforgettable: Alex, Aileen, Areti, Nikos, Viky, Spiridou, Jos, Lydia, Giorgos, Dimitris the 1st, Yvonne. Also thanks to Leuki, Qanasi, Taso and Aggelo for all their support and friendship. I would like to thank all my relatives (too many ☺) for moral support and special thanks to Sotiris Tsantarliotis for being always a friend and Vasiliki Koukouviki for supporting me in some difficult years.

My family (Giorgos, Eleni, Sofia, Evaggelia (sporos)) have been supporting and helping me for all my life. They have always helped and believed in me, giving me strength in the hardest times. They deserve a very big THANK YOU for all what they have done for me.

Makrina Totsika has been the closest person I had during this Ph.D. She has seen and endured with me the ups and downs of this course. I want to thank her for supporting me, and for all the moments we have been through together. Thank you Makrinako !!!

For the 2nd chapter all staff at MLURI's Sourhope Research Station, especially Gavin Rogerson, are thanked for their time and effort in managing the goats and collecting the data. This project is funded from the Scottish Executive Rural Affairs Department, the Scottish Development Agency and the European Community is gratefully acknowledged

Finally I would also like to thank State Scholarship Foundation of Hellas, MLC, A.S. Onassis Public Benefit Foundation and my family for their financial support in various periods of this Ph.D.

List of Publications

Vagenas, D., Jackson, F., Russel, A.J.F., Merchant, M., Wright, I.A. and Bishop, S.C. 2002. Genetic control of resistance to gastro-intestinal parasites in crossbred cashmere-producing goats: responses to selection, genetic parameters and relationships with production traits. *Animal Science*. **74:** 199-208

Vagenas, D. and Bishop, S.C. 2002. Modelling the joint effects of genotype and protein intake on resistance to gastrointestinal parasite infections in sheep. *7th World Congress on Genetics Applied to Livestock Production. Montpellier, August 19-23*

Abstract

The aim of this thesis is to explore some of the quantitative genetic aspects of resistance to gastrointestinal nematode parasites in small ruminants. The focus is on animals in temperate areas such as Northern Europe and consequently on the parasite species which poses the main production losses in this area, *Teladorsagia circumcincta.*

Most of the published genetic parameter estimates in the literature for resistance to nematode parasites are for sheep, while there is a distinct lack of estimates for goats. A genetic analyses of parasitological and production data from a cashmere goat population (kids) was performed. The data were collected over a time span of four years from a farm in Scotland. The heritability of the indicator trait of resistance faecal egg count (Fec) was estimated to be 0.17 while that of the mean of several measurements was 0.32. The heritability of fibre traits was in excess of 0.5, while the heritability of live weight was 0.22. The genetic correlations between Fec and the production traits were slightly positive but not significantly different from zero. The phenotypic correlations were very close to zero.

Fec data collected over four years from a commercial flock of Scottish Blackface in Scotland were analyzed using random regressions analyses to estimate the genetic and phenotypic parameters of the flock and how they change over time. The random regression model gave at least as good description of the data as univariate models fitted at individual time points. The added benefit of random regression analysis was that it allowed heritabilities and correlations to be interpolated for time points when data were not available, thereby enabling sampling time strategies to be determined. Genetic correlations between samples taken from 14 to 24 weeks of age were all greater than 0.8.

The distribution of Fec in sheep has been found to generally fit the negative binomial distribution, with a small proportion of animals shedding most of the parasites. This fact could be utilised for separating or culling the most parasitised animals, as an immediate control measure. By means of computer simulation, a scenario of exploiting this particular distribution of Fec combined with selection over ten years in a closed flock was examined, *in silico*. Different management scenarios and different culling/separation scenarios were explored. The impact on lamb performance after ten years of separating the worst animals based on Fec did not exceed a 4% improvement in live weight in any scenario. The effect of culling was higher but there is a trade off by the fact that there is a profit loss due to the loss

of animals. Thus there is relatively low merit in using, additionally to selection, separation/culling to help control the impact of nematode parasites.

Protein supplementation has been proposed as a means of helping to reduce the impact of gastrointestinal parasites. The interaction of genotype and nutrition and the effect of different levels of protein supplementation on estimated genetic and phenotypic parameters for a flock of lambs were examined using computer simulation, for artificial and natural challenge scenarios. In the artificial challenge scenario the correlations between Fec and production traits became stronger as dietary protein level was reduced and the parasitic challenge was increased. There was little discernible pattern for natural challenge. It may be concluded that the predicted genotype x environment interaction is of little practical significance with respect to challenge level and dietary protein content.

These results contribute to our understanding of the genetics properties of the resistance to gastrointestinal nematodes of small ruminants. They will allow more effective design of breeding strategies for nematode resistance in small ruminants. In particular, this thesis has demonstrated that breeding schemes for improving nematode resistance in small ruminants are predicted to be robust with respect to a) age of sampling b) nutritional regime (protein) and c) management (culling or separation).

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1. Breeding for resistance to nematode parasites

1.1 Parasite diversity and importance

In ancient Greek "parasite" means the person who is eating at another's Table. In modern languages parasite has a different meaning, the common feature of which is that the parasite is living at the expense of somebody else (for example in Greek, Italian, English, and Spanish). In the scientific terminology parasite is defined as: "a plant or animal that lives upon or within another living organism at whose expense it obtains some advantage" (Blood and Studdert, 1988). It has been estimated that at least two thirds of the one million five hundred thousands species of plants and animals that have been identified to date are parasites. They can be as small as 10^{-12} m and as long as 30m (Ackerman, 1998).

Many different classifications can be followed for parasites. However two major categories have been used for classification of parasites:

Ectoparasies: Parasites living on the surface of the host's body (Blood and Studdert 1988, Matthews 1998).

Endoparasites: Parasites that live within the body of the host (Blood and Studdert, 1988). Nematode parasites of the gastrointestinal tract belong to this category.

However exciting their study and their relationship with the host may be, nematodes represent a major source of loss in the animal production industry. Many studies in different countries have demonstrated that the cost of nematodiasis is considerably high. For example, in Australia Piper and Barger (1988) estimated that the total loss caused by helminth parasites in the sheep production industry was A$450M per year when the total value of wool exports was A$2400M. A more recent estimate is $220 million per year (Sandeman and Warner, 2002). These authors predict that the worm control cost is expected to rise four

to five fold within ten years. For the UK industry, Bishop (1996) estimated that the production losses due to infections alone were £30 - 40M. Inclusion of cost for drugs, veterinary care and labour would increase the losses further. There are no estimates known for the production loss caused by gastrointestinal parasites in goats. The UK industry is very small compared to the sheep production industry. This is reflected, as will be seen in this introduction, in the fact that there are many more studies for sheep than goats. The main focus will thus be on what is known for sheep and wherever there is information for goats this will be given. Most of the introduction refers to Strongyles species. When information is given for Nematodirus it will be explicitly stated.

Goats are less resistant than sheep to parasite infection (Le Jambre and Royal, 1976) and there are indications that parasites harboured by goats develop faster resistance to anthelminthics than do parasites harboured by sheep (Kettle *et al.* 1983). This will have an adverse effect on sheep grazing the same pastures with goats. Therefore, there is a need for more experiments involving goats, which will advance our understanding of the genetic mechanisms involved in the interaction between host and parasite in small ruminants.

The reduction in profitability or production of sheep is due to a number of causes:

a) Mortality, which includes the capital cost of replacement. This may not seem important in temperate regions, but it is very important in tropical and subtropical regions where the death of an animal, especially a large animal like buffalo and cattle, can be a disaster for the farmer (Waller, 1997).

b) Quantitative and qualitative reduction in liveweight gains, wool growth and reproductive efficiency. In a study involving grazing lambs, Coop *et al.* (1985) found that

gastrointestinal parasitism can result in a reduction of liveweight gain of 30 to 40%. Their study indicated that lambs of 20 to 25kg exposed to moderate intakes of *Teladorsagia circumcincta* larvae could take 4 to 7 weeks longer to reach the target weight of 36 to 38kg than lambs exposed to low numbers of *T. circumcincta* larvae.

c) Increased costs of production arising from the use of anthelminthics and the labour involved in their administration (Windon, 1990).

d) Costs incurred by avoiding pastures that are highly contaminated. It is well understood that returns to sheep farmers continue to decrease, and farmers are expected to farm more sheep per labour unit with decreased costs per sheep. Important recurrent costs are associated with flock health programmes and farmers are faced with the necessity of minimising costs whilst maintaining acceptable levels of health control (Raadsma *et al.* 1998)

e) Consumer demand for animal products free of chemical residues

1.2 Life cycle of nematode worms

Nematodes are cylindrical and taped at each end. Their bodies have a thick cuticle (Matthews, 1998), and they have a mouth and an anus. Although there are a few nematode species that are hermaphrodite or parthenogenetic, most have separate sexes (Matthews, 1998). The basic nematode life cycle has six stages: the adults, the eggs and four larval stages. The larvae are still recognisably nematodes but lack the sexual organs and other adult features. The second (L_2), third (L_3) and fourth (L_4) larval stages and the adults are proceeded by a cuticular moult when all the cuticular structures including the lining of the

bucal cavity and the rectum are replaced (Matthews, 1998). *T. circumcincta* also has a L5 stage.

Specifically for gastrointestinal parasites of small ruminants, the eggs are laid by the adult worm inside the host. These eggs are then passed out of the sheep with the faeces, where they continue to develop on the pasture. The L1 breaks out of the eggs and feeds on bacteria in the faeces, then develops into the L2 and the L3. This last larval stage is the infective one, which is ingested by the host during grazing. It develops into the L4 and settles at the chosen site of the gut where it matures.

1.3 Issues concerning anthelminthic drugs

The most widely used approach of controlling parasites is through the administration of anthelminthics. In the last decades, however, concerns have increased regarding their usage for several reasons which will be examined in this section.

In a parasite context, resistance is the situation where parasites usually affected by a given dose or concentration of a compound are no longer affected. Resistance is inherited (Sangster, 1999) and reversion of resistant strains to susceptibility should not be expected and has not been observed (Waller, 1999). In many countries resistance to anthelminthics has reached alarming levels. For example, in South Africa around 90% of sheep farms have parasite strains resistant to compounds from at least one anthelminthic group and approximately 65% of farms now have to confront the problem of multiple anthelminthic resistance (van Wyk *et al.* Web site). In South Africa there have been a number of cases where farmers were forced to abandon sheep farming because of failure to control worms by chemotherapy (Waller, 1997). Sandman and Warner (2002) predict that the levels of resistance are such that in Australia none of the current drugs are likely to be effective

against the major intestinal worm in the next ten years. As a consequence, according to these authors, sheep production will no longer be economic in the northern half of the current geographical range. A sample of other countries where resistance to anthelminthics has been observed are Uruguay, Brazil, Paraguay, Malaysia, Fiji, India, Australia and New Zealand (Waller 1999, Barger 1999, Leathwick *et al.* 2001).

Mitchell *et al.* (1991) found that 24% of the sheep farms in Scotland had parasites resistant to benzimidazole present. A survey by Hong *et al.* (1992) found that resistant strains to benzimidazole were present in 47% of the sheep farms in south England. In a similar survey in sheep and goat farms in England and Wales, Hong *et al.* (1996) found that there was a spatial difference in the distribution of farms where resistance to anthelminthics was detected. In the Northeast resistance to anthelminthics was detected in 15% of the farms in contrast with 44% of the farms in the Southwest. In the same study, resistant strains of nematodes were detected in 65% of the goat farms. These percentages are likely to increase due to non-implementation of farming practices that could slow down the process of nematodes developing resistance to drugs (e.g. correct dosing, minimal treatments, rotation of anthelminthic types, clean grazing etc.) (Coles, 1997).

Two types of multiple anthelminthic resistance have been defined (Sangster, 1999):

1) Side resistance, which is the phenomenon where parasites resistant to one drug of a chemical class are also resistant to others in the same class.

2) Cross-resistance, which is the resistance to unrelated drugs.

There is evidence of both side resistance and cross-resistance in gastrointestinal parasites. (Sangster, 1999). It should be noted that it seems unlikely for the next few years that anthelminthic treatment will cease; ideally it would be used strategically in conjunction with other measures described below to control parasites.

Development of new anthelminthic drugs might be an answer to the developing resistance. However, high developmental costs in identifying new anthelminthics and stringent government regulations involved in the registration of new drugs mean that private sector companies are reluctant to invest in the development of new generation anthelminthic drugs (Matthews, 1998). This is even more so because there are potentially more profiTable areas for investment such as human and companion animal disease control and cosmetics. Governments are also reluctant to invest in the development of new drugs. Therefore, in recent years there has been a reduction in the search for anthelminthic drugs.

Another important issue, which should be taken into account, is the impact of the anthelminthic residues on the environment. There is an increasing community awareness of environmental issues (contamination), but there is also another impact of the anthelminthic residues on the environment that affects the epidemiology of parasites: there are indications that they kill some of the non-target species such as dung beetles (Schillhorn van Veen, 1999). There is evidence that dung beetle activity is associated with reduction in infective larvae recovered from faeces (and the herbage surrounding them) for livestock that produce large faecal masses, like cattle and horses (Waller and Faedo, 1996). Thus, it could be considered that anthelminthics may have a negative impact on other biological enemies of nematode parasites along with the aesthetic change of the landscape due to the reduction of decomposition of faeces.

The most crucial fact from the issues described above is that parasites have the ability to adapt to control measures imposed against them. Once, it was expected that anthelminthics would act as an absolute drug against parasites in the same way that antibiotics were considered an ultimate control against bacteria. Nowadays it has become evident that this is not the case. Only by combining measures that act via different mechanisms will the problem of parasitism be minimised. It is understood that the parasites have the potential to adapt so as to overcome control measures, but the more sophisticated and multi-action these measures are the more difficult it will be for parasites to adapt. Thus the best way of controlling nematodes would most probably be to design a protection scheme, which is easy for the layman to implement, but at the same time prevents the parasites from evolving and adapting to it. For achieving this the option of combining measures, which come from different disciplines, should be explored.

1.4 Alternative Approaches for Controlling Nematode Parasites

Alternative or complementary methods that have been sought to control nematode parasites will now be described. According to Barger (1999) the most important parameters determining the challenge the animals will face are larval availability and the survival rate of the larvae on the pasture, and the climatic requirements for egg hatching and larval development.

Several alternative approaches to the problem of controlling parasites have been proposed in recent years. These are given in Table 1.1 with a brief description.

Table 1.1 Alternative approaches for reducing the effects of gastrointestinal parasites on the productivity of animals

Technique	Description
Grazing management	Management of the pasture, rotational grazing to minimise larval challenge
Vaccination	Vaccination of animals
Biological control of parasites	Use of organisms which have detrimental effects on parasites, such as nematofagus fungi, dung beetles
Selection for resistant hosts	Genetic selection of hosts for lower worm burden
Nutrition supplementation	Protein supplementation of the host to enhance immune response

Grazing managment. This technique attempts to tackle the problem of nematodes via the management of the pasture. The aim is to minimize the exposure of susceptible animals to highly infectious pastures, for example by rotational grazing between small ruminants and cattle. This rotational grazing is based on the fact that cattle and small ruminants are infected by different nematode species and thus one species could 'sweep' the larvae which infect the other animal species. Alternatively the pasture is divided into parts, which are grazed after long spells and result in the majority of the infective larvae dying on the pasture, before susceptible animals graze the pasture again (Waller, 1997).

Vaccines. The development of vaccines against gastrointestinal nematodes that are feasible to use on a large number of animals has not been very successful. At the moment, there is no commercially available vaccine against gastrointestinal nematodes (Hein *et al.* 2001).

Biological control of the parasites. Living organisms that in some way reduce the numbers of the infective larvae of the gastrointestinal nematodes are dispersed on the pasture or administered to the lambs (Waller and Faedo 1996, Hein *et al.* 2001). One category includes dung beetles and earthworms, which destruct the environment where larvae are found. Their ability to reduce the number of the larvae has been found to be limited. Furthermore, recent evidence indicates that dung-burial from beetles might actually provide a protective reservoir for infective larvae (Waghorn *et al.* 2002). The other category includes microfungi that either entrap nematodes or infect them, with the latter likely to be more effective. However, there are problems with the administration of the micro-fungi to the animals and they might have unknown consequences on the field ecology (Hein *et al.* 2001).

Selection for resistant hosts. This method is the main focus of this thesis and will be further explored in the following chapters.

Nutrition supplementation. There is evidence that nutritional supplementation, especially protein, can have a beneficial effect in alleviating the effects of parasitism (Sykes and Coop 2001, Coop and Kyriazakis 2001), allowing the animal to have a more effective immune response. Thus it has been proposed that protein should be supplemented to animals that are parasitised or in a strategic manner to classes of livestock such as recently weaned lambs or periparturient ewes that are likely to be especially susceptible to parasites.

1.5 Breeding for Resistance: general principles

A distinction has to be made at this point between infection and disease. Infection is the colonization of a host animal by a parasite (Bishop, 2002); disease describes the side effects of infection by a parasite (Bishop *et al.* 2002). Disease resistance is defined as the ability of

the host to resist infection or control the parasites' lifecycle; therefore, resistant animals harbour fewer parasites than susceptible animals (Woolaston and Baker 1996, Raadsma *et al.* 1997a, Raadsma *et al.* 1998). Furthermore, resistant animals transmit fewer parasites, i.e. eggs, which means reduced pasture contamination (Stear *et al.*1997a, Bishop and Stear 1999).

The host may achieve enhanced resistance to internal parasites by a number of ways (Raadsma *et al.* 1998, Anonymous, 1994):

a) Reduced establishment of incoming larvae
b) Arrest or delayed development
c) Accelerated expulsion of adult worms
d) Reduced fecundity of female worms

Specifically for *T. circumcincta* infections of lambs the key features of resistance are the following (Stear *et al.* 1996, Stear *et al.* 1997a, Stear *et al.* 1999):

1) Resistance is acquired and not innate as is implied by the lack of detecTable genetic variation in the first months of a lambs life.
2) Worm length is positively associated with worm fecundity
3) Worm length and hence fecundity declines as worm number in sheep increases
4) IgA appears to be a major mechanism regulating worm length
5) Lambs can apparently control worm length but not worm number

Older animals have substantially fewer parasites than lambs due to acquisition of effective immune responses that reduce worm numbers, possibly through immediate hypersensitivity reactions against incoming third stage larvae (Stear *et al.* 1999). Most of the parasites in a

population of sheep are harboured in animals that either do not have a properly developed immune system or are under some kind of stress. Typically, this includes lambs and periparturient ewes.

The overall hypothesis of Stear *et al.* (1999) is that immunity for *T. circumcincta* develops in two stages:

1) Firstly animals develop the ability to control worm growth and fecundity

2) Subsequently they develop the ability control worm numbers

It should be noted that two other approaches have been proposed for selection for reduced economic effect of parasites on small ruminant production, namely breeding for resilience and breeding for reduced treatment cost. Breeding for resilience is effectively the same thing as breeding for production traits under parasitism. Breeding for reduced treatment cost does not properly address the problem and has been found to have a low heritability (Bisset *et al.* 1994, Bisset *et al.* 1997). The methods mentioned in this section all have a common disadvantage: animals have to be exposed to parasites before their superiority to the rest of the flock can be demonstrated.

1.6 Measuring Resistance to Nematode Infection

The objective in measuring resistance to nematode infection is to estimate the number or mass of the worms (i.e. worm burden) in an adult animal. However, this is not currently possible for live animals since it would involve the slaughter of the animals to enable the number of worms in the gut to be counted. Therefore a trait correlated with worm burden should be used.

Different traits have been proposed as indicators for resistance to gastrointestinal parasites, for example dagginess (daggs are defined as locks or staples of wool in the crutch that are heavily fouled with caked faeces), anaemia and blood fructosamine concentration. Most of these traits have at least one of the following problems: a) they are weakly correlated with worm burden; b) they can be affected/caused by conditions other than parasitism; c) they have a low heritability (Windon 1990, Beh and Madddox 1996, Raadsma *et al.* 1997a).

The most suiTable indicator trait found thus far for resistance is the number of parasite eggs in a sample of the animals' faeces (faecal egg count, Fec). Field and experimental studies (Windon 1990, Baker *et al.* 1991, Bishop *et al.* 1996, Stear *et al.* 1997a) have shown that faecal egg count is a variable trait in various breeds of sheep infected by different parasite species. However, the relationship between faecal egg count and parasite burden is not linear, at least for *T. circumcincta* (Bishop and Stear, 2000).

Faecal egg count is a simple trait to measure. It is extremely variable and moderately heritable and thus amenable to reasonably rapid genetic progress. For other traits, which can be used to indicate resistance, heritability estimates are generally no higher than those for faecal egg count and genetic correlation of these indicators with faecal egg count and worm burden are substantially less than unity. Therefore, they are less efficient selection criteria than faecal egg count. The role of these traits will only be important if they can be shown to reflect additional components of genetic variation in resistance not covered by faecal egg count (Raadsma *et al.* 1998).

Faecal egg count is a function of both the number of the female parasites and their average fecundity (Bishop, 1996) and so it is affected by factors affecting both components. Differences in adult worm burden can arise from differences in grazing behaviour or

differences in the efficiency of host immunological responses (Stear *et al.* 1996). It has been found that fecundity of the female parasites decreases dramatically as the parasite population increases (Bishop and Stear, 2000). This is known as a density dependent effect (Anderson and May, 1992) and can influence the relationship of faecal egg count with parasite burden, at least for *T. circumcincta* (Bishop, 1996).

Faecal egg counts differ with respect to many other traits in the shape of their distribution amongst individuals. The distribution of faecal egg count is heavily skewed or over-dispersed, with a few individuals having high faecal egg counts compared with the majority of the hosts which have relatively low faecal egg count (Sreter *et al.* 1994a, Stear *et al.* 1995, Stear *et al.* 1998) and it often fits the negative binomial distribution. A rule of thumb is that 20% of the animals host 80% of the total parasites in the flock (Bishop and Gettinby, 2000). This over-dispersion has also been observed in goats (Hoste *et al.* 2001). Although the over-dispersion of faecal egg count (and subsequently worm burden) is relatively well documented, no way of exploiting this phenomenon has been rigorously explored. For example, what would happen in the short to medium term, if a given proportion of the less resistant animals and hence more heavily contaminated were removed from the flock? Questions like this should be addressed and further explored.

Stear *et al.* (1999) reported a low heritability (0.14±0.10) for worm burden in contrast with high heritability for worm fecundity (0.62±0.20) in six month old lambs (the heritability in sheep of different age is unknown). It was suggested that the genetic variation in worm burdens of six months old lambs naturally infected predominantly with *T. circumcincta* is under limited genetic control (see subsequent section) unlike differences in worm fecundity. Selection of hosts that are resistant may result in reduced parasite fecundity and subsequently

a reduced parasite population on the pasture, and risk of infection, rather than directly reducing the parasite burden in the host.

As mentioned before, the fecundity of the female parasites decreases as the number of the parasites increases (density dependence effect). The pathogenesis of *T. circumcincta* is influenced both by the size and the number of the individuals in the parasite population. In a study with lambs infected by *T. circumcincta* Bishop and Stear (2000) found a decrease in worm length at high intensities of infection. According to their results strong density dependent effects are seen in years with high mean worm burdens, and can result in low faecal egg count. In these years it is worm fecundity that tends to determine faecal egg count for individual sheep. In contrast, in years where there is an absence of density dependent effects, worm burden tends to be more important than worm fecundity in determining faecal egg count. It is suggested that density-dependent effects have an impact on pathogenesis and these may minimise the pathogenic effects in heavily infected individuals, resulting in benefits to both the parasite and the host.

1.7 Heritabilities of faecal egg count

In this section published estimates of the heritability faecal egg count for a range of breeds are summarized and discussed. Many of the estimates are taken from the review of Raadsma *et al.* (1997a) and Eady *et al.* 1996.

The estimates of heritability in Table 1.2 are based on natural, mixed parasite challenge. The main parasite species of the challenge differ according to the country in which the study took place. In studies conducted in Europe (temperate regions) the main parasite species is *T. circumcincta*. In tropical-subtropical regions (Australia, Kenya etc.) the predominant species are *Haemonchus contortous* and the genera *Trichostrongylous*. The difference in the parasite

species might, partly, account for differences in the estimates of the phenotypic and genetic parameters from different studies, although the standard errors of the estimates are generally substantial.

Table 1.2. Summary of published estimates of heritabilities (s.e) for faecal egg count. Natural mixed challenge

Breed	Age (months)	Counts Fec	Heritability	Reference	
Romney	5-8	1	0.34 (0.19)	Watson *et al.* 1986	New Zealand
Romney	5-8	2	0.53 (0.15)	Baker *et al.* 1991	New Zealand
Romney	5-8	1	0.27 (0.07)	Bisset *et al.* 1992	New Zealand
Romney	5	1	0.13 (0.07)	McEwan *et al.* 1992	New Zealand
Romney	4	1	0.39 (0.13)	Morris *et al.* 1993	New Zealand
Romney	6	1	0.46 (0.14)	Morris *et al.* 1993	New Zealand
Romney	<9	2	0.28 (0.02)	Morris *et al.* 2000	New Zealand
Red Maasai, Dorper	10	2	0.20 (0.08)	Baker *et al.* 1994	Kenya
Polish Longwool	6 and 8	2	0.28 (0.16)	Gruner and Lantier, 1995	Poland
Polish Longwool	6,8	2	0.20-0.33 (0.04)	Bouix *et al.* 1998	Poland
Merino	6 and 12	2	0.42 (0.14)	Cumins *et al.* 1991	Australia
Merino	10 and 22	1	0.16 (0.12)	Eady *et al.* 1996	Australia
Scottish Blackface	3-6	4	0.33 (0.15)	Bishop *et al.* 1996	UK

In Table 1.3 a summary of published heritabilities conducted using artificial challenge are shown.

Table 1.3. Summary of published estimates of heritabilities (s.e, where available) for faecal egg count. Artificial challenge

Parasite species	Breed	Age	Counts Fec	Heritability	Reference	Country
Haemonchus contortus						
	Romanov	6-10	6	0.55	Gruner and Lantier 1995	France
	Merino	18	1	0.23 (0.13)	Piper 1987	Australia
	Merino	4-5	2	0.30 (0.10)	Albers *et al.* 1987	Australia
	Merino	4-5	1	0.29 (0.03)	Woolaston and Piper 1996	Australia
	Merino	6-8	1	0.30 (0.13)	Eady *et al.* 1996	Australia
	Hungarian Merino	6-7	4	0.49 (0.17)	Sreter *et al.* 1994b	Hungary
Trichostrongylu colubriformis						
	Merino	4-5	5	0.41 (0.04)	Woolaston *et al.* 1991	Australia
	Merino	5-7	5	0.38 (0.04)	Woolaston and Windon 2001	Australia
	Merino	5	3	0.21 (0.06)	Woolaston and Windon 2001	Australia
	Merino	7	3	0.35 (0.03)	Woolaston and Windon 2001	Australia
	Merino	5-13	1	0.20 (0.11)	Eady *et al.* 1996	Australia

The first thing that is apparent from these Tables is that the heritability of the mean of more than one measurement is generally higher than that of a single measurement. This is predicted from quantitative genetics theory as the variance specific to the animal is reduced, resulting in a lower overall environmental variance. As can be seen from Tables 1.2 and 1.3, the heritability of faecal egg count is generally between 0.2-0.4. For very young lambs (1-2 months) the estimated heritability is effectively 0 (Bishop *et al.* 1996). Moreover, the heritability for the mean of more than one measurement can be over 0.4. In summary, faecal egg count is a moderately heriTable trait with potential for genetic improvement.

In general, there are not great differences between the estimates of heritability for natural and artificial challenges. Studies in Australia, New Zealand and France have shown that there is a high genetic correlation ($r_g > 0.9$) between artificial challenge (single nematode species)

and natural pasture challenge (multi-species challenge) (Baker 1999, Elsen *et al.* 1999, Gruner and Lantier, 1995).

1.8 Correlations between live-weight and resistance.

The phenotypic correlation between faecal egg count and productivity traits seems to be weak and in most cases close to zero (Douch *et al.* 1995, Baker *et al.* 1991, Bisset *et al* 1992, Eady *et al.* 1994, Bishop *et al.* 1996). In the following section some estimates of genetic correlations from studies in Europe, New Zealand and Australia will be given.

Published estimates of genetic correlations between faecal egg count and growth rate from the literature for Europe are mostly negative, i.e. favourable. Bishop *et al.* (1996) found a strong negative correlation (magnitude of -0.8) (r_p in region of –0.01) between faecal egg count and production traits in lambs naturally infected, predominately with *T. circumcincta.* This suggested that resistant to gastrointestinal parasites is probably an important determinant of growth rate in this environment. More recently, Bouix *et al.* (1998) also found a strong negative correlation (-0.61) between faecal egg count and live weight, estimated from a lamb population in Poland. In the UK the average of published and unpublished genetic correlations between faecal egg count and live-weight is –0.42 (Bishop, 2001).

In New Zealand MacEwan *et al.* (1992, 1995) estimated a moderately positive genetic correlation between production traits and faecal egg count (in the region of 0.40) whereas Douch *et al.* (1995) estimated a genetic correlation of –0.13 (r_p = -0.15) between faecal egg count and live-weight. The genetic correlation between faecal egg count and growth rate was stronger, -0.30 (r_p = -0.12). Again it should be pointed out that the predominant species in various experiments differ depending on the major parasite species in the country of study,

although these results also indicate inconsistency between studies done within a single country.

Estimates of genetic correlations between faecal egg count and live-weight from Australia vary. Eady *et al.* (1994) estimated a genetic correlation of 0.19 between weaning weight and faecal egg counts, and a genetic correlation of 0.12 between faecal egg count and hogget live-weight (no r_p given). More recently Eady *et al.* (1998) estimated genetic correlations of –0.20, -0.18, -0.26 (r_p< -0.10 in all cases) between faecal egg count and weaning, ten month and sixteen month weight, respectively.

What can be seen from the previous paragraphs is that in Europe there is consistency in the estimates of genetic correlation between faecal egg count and live-weight, insofar that they are always negative. In Australia the estimates range from slightly unfavourable to slightly favourable. In New Zealand both moderately favourable and moderately unfavourable estimates have been observed. The predominant species in Europe is *T. circumcincta* whereas that in New Zealand is *Trichostrongylous* spp. (*T. circumcincta* also present) and in Australia *Trichostrongylous* spp. and *Haemonchus contortus*. These observations stress the need for caution when making assumptions about the genetic correlation between production traits and resistance to nematodes, as they appear to differ considerably between studies.

Closely related to the above two parameters is the repeatability of faecal egg count and the number of measurements taken per animal, as the heritability rises as the number of measurement taken per animal increase (Falconer and Mackay, 1997). The repeatability of faecal egg count varies from zero to 0.86 (under field conditions) and it is sensitive to changes in management (Kelly and Gray, 1995). The genetic correlation between two different measurements appears to be not significantly less than unity (in lambs older than

three months of age) indicating that each faecal egg count is expression of the same underlying trait (Bishop *et al.* 1996).

1.9 Genetic parameter estimates for goats

For goats there have been fewer experiments enabling the estimation of genetic and phenotypic parameters, and these have yielded generally lower estimates of these parameters compared with sheep. The published estimates are given in Table 1.4.

Table 1.4 Summary of published estimate of heritabilities (s.e.) for faecal egg count in goats Natural challenge.

Age	h^2 s.e	Reference
weaners	0.04 (0.03)	Woolaston *et al.* 1992
Adult	0.08 (0.06)	Woolaston *et al.* 1992
weaners	0.11 (0.11)	Baker *et al.* 1998
6	0.03 (0.03)	Baker *et al.* 2001
2	0.08 (0.07)	Baker *et al.* 2001
4.5	0.26 (0.15)	Baker *et al.* 2001
14	0.08 (0.12)	Baker *et al.* 2001
3	0.37	Mandonnet *et al.* 2001
4	0.14 (0.05)	Mandonnet *et al.* 2001
6	0.17 (0.05)	Mandonnet *et al.* 2001
8	0.17 (0.05)	Mandonnet *et al.* 2001
10	0.33 (0.06)	Mandonnet *et al.* 2001

It should be noted that the heritabilities at four and ten months of age given by Mandonnet *et al.* (2001) were estimated for the mean of two measurements taken one week apart. Assuming that they represent the same infection, these estimates of heritability are higher than those which would have been obtained on the basis of one measurement, due to the averaging of sampling effects.

1.10 Quantitative Trait Loci (QTL) and major genes

1.10.1 QTL detected by linkage studies

MacDonald *et* al. (1998) completed a joint analysis of two half-sib, Texel-Coopworth backcross pedigrees (n=131 and 143 progeny respectively in a study searching for a QTL affecting dagginess in sheep. The above authors found evidence for a QTL affecting this trait. In their experiment the animals were also measured for faecal egg count but no evidence for a QTL affecting faecal egg count was found. They concluded that there was evidence that a QTL could affect these traits independently.

Beh *et al.* (2001) carried out a linkage scan of the genome of 1029 sheep in six half-sib families for QTLs affecting faecal egg count. The families were offspring of sires from two divergent lines of sheep selected for response to challenge with *T. colubriformis*. A total of 472 lambs from the extremes of the distribution of faecal egg count were genotyped using 133 microsatellite markers covering all 26 autosomal chromosomes of sheep. These authors found six chromosomal areas that appeared to have a significant effect on resistance (faecal egg count) to gastrointestinal parasites (in chromosomes 1, 3, 6, 11, 12) in sheep but nothing significant at the genome wide level. It should be noted that the parasite studied by Beh *et al.* (2001) was *T. colubriformis*, which may invoke different host defence mechanisms than *T. circumcincta*, which is the main parasite species in Europe.

Shay *et al.* (2002) carried out a study for the detection of QTLs affecting resistance to nematode parasites, using animals from an F2 generation of Suffolk (susceptible) and Gulf Coast Native (resistant) breeds. Preliminary results indicated that QTLs for resistance might be present in chromosomes 1, 3 and 19. Interval mapping using all fifty markers genotyped

indicated that the most probable position for a putative QTL covers the central region of chromosome 1.

1.10.2 Major genes inferred by segregation analyses

McEwan and Kerr (1998) undertook a segregation analysis, using a mixed inheritance model, which suggested that a dominant autosomal gene for host susceptibility was affecting strongyle faecal egg count values in the population which they studied. The animals were naturally challenged, from ten weeks of age, and monitored till the average faecal egg count in a sample of lambs was 1000 eggs/g (main species *T. circumcincta* and *T. colubriformis*) when they were drenched. The above process was repeated twice and measurements were taken at approximately 18 and 26 weeks of age. Measurements were taken at the same time for *Nematodirus*. The estimate of the effect of a single allele affecting susceptibility of the host was 0.79 log (eggs/g) and a dominance effect of 0.96 log (eggs/g). The model that fitted the data best had susceptibility to *Nematodirus* as a recessive effect. The effects of a single allele were 0.64 log eggs/g and the dominance effect was –0.68 log eggs/g. From this study it was not possible to infer if the putative QTLs affecting susceptibility to *Strongyles* and *Nematodirus* were the same.

Meszaros *et al.* (1999) did a segregation analysis of data from a flock known as the 'Golden Ram' flock. Segregation of a major gene had long been suspected in this flock, but early attempts to detect a major gene were inconclusive (Woolaston, 1990). In this study however, a major gene effect responsible for resistance to *H. contortus* was inferred, accounting for approximately one third of the total genetic variance of faecal egg count.

1.10.3 Major Histocompatability Complex

The Major Histocompatability Complex (MHC) is an assembly of genes encoding molecules that are intimately involved in the control of immune response and disease resistance (Rothschild *et al.* 2000). The MHC is usually subdivided into three regions termed MHC class I, II and III (Hein, 1997). The majority of cells in mammals have class I MHC molecules but some specialised cells including macrophages and some B-lymphocytes have class II MHC molecules (Matthews, 1998).

There have been conflicting results in the literature for the association of class I genes of the MHC and resistance with nematode parasites. Outteridge *et al.* (1985, 1986, 1988) reported an association between class I antigens of MHC and increased resistance to *T. colubriformis*. This was also the case in a study with lambs infected with *T. circumcincta* (Raadsma *et al.* 1997a). Studies with sheep infected with *H. contortus* have given conflicting results (Raadsma *et al.* 1997a, Beh and Maddox 1996). Thus selecting animals on the basis of particular class I MHC antigens is not recommended (Raadsma *et al.* 1997a).

Janβen *et al.* (2002) performed a study to estimate association between parameters of parasite resistance and six genetic markers located in the MHC complex in sheep. They used five Rhonschaf half sib families (n=468) and individuals were infected orally with 5000 larvae of *H. contortus*. They found a significant association between resistance to parasites and three of the markers used. Allele A of one marker was associated with positive effect of 7% on the haematocrit level while the allele B of the same marker was associated with a negative effect (-19%). Allele C of the second marker was associated with reduced IgL level(16%). Allele C of the third marker was associated with reduced faecal egg count (6%).

Studies with Scottish Blackface sheep have found an association between type II antigens and resistance to nematode parasites (Stear *et* al. 1996b, Schwaiger *et al.* 1995, Buitkamp *et al.* 1996). However, other researchers have not been able to confirm this association (Hulme *et al.* 1993, Blattman *et al.* 1993, Crawford *et al.* 1997). Possible reasons for this are the following:

1) The experimental power of the half sib-pedigrees used
2) Different parasite species used in the experiments and for which the underlying mechanisms of resistance might be different.
3) The kind of markers used might not be very informative.

This lack of association was also found in a study of Polish Heath sheep (Charon *et al.* 1998). The above conflicting results led Raadsma *et al.* (1997a) to suggest that until the role of this marker in other breeds and with parasites other than *T. circumcincta* and *H. contortus* is fully understood it should not be used for selective breeding.

1.11 Theoretical studies – Simulations and Modeling

In general, the genetic improvement of traits describing disease resistance has to take into account a fundamental phenomenon: the disease epidemiology. Disease epidemiology is the key factor which differentiates disease resistance traits from productivity traits. From the epidemiology, one may describe how an animal's health will affect the health status of other animals. It is a difficult task to quantify these interactions except in costly long-term experiments, which are complicated and difficult to manage. Furthermore, the actual infection will pose an ethical barrier. It may not be ethically acceptable to expose animals to an infection on the scale required for studying the quantitative genetic aspects of the population, irrespective of whether it is bacterial, viral or parasitic. The last, but certainly not

the least factor, is the difficulty of funding such studies. Therefore, the long-term effects of different control strategies against parasites, including breeding for resistance, have been studied using computer modeling techniques. The advantages of computer modeling are numerous. They can be summarized as cost effectiveness, the fact that the same scenario can be replicated many times and that the scenario setting can be modified easily, and there are no associated ethical barriers. These models try to mimic what would happen in field conditions, at least in some aspects. The major disadvantage of models is that they are a description of nature as we perceive it, and thus may be a poor description of a natural process.

There have been many models describing parasite epidemiology and the factors affecting it and in this section those examining the effects of parasitism on productivity will be described. All the models were based on field data and calibrated so that, in circumstances for which data were available, they behaved properly insofar as they reproduced results typical of field data.

Barger (1989) was the first to study the effects of host genotype on disease epidemiology. The effect of the resistant animals on parasite epidemiology was to reduce the amplitude of the seasonal fluctuations creating a loop whereby animals with greater resistance lead to less pasture contamination. Subsequently the challenge to the animals could be lower and both resistant and unselected animals could benefit from this reduced pasture contamination.

Leathwick *et al.* (1992) explicitly incorporated production losses due to infection of gastrointestinal parasites, which they modeled as a function of both cumulative worm burden and cumulative larval challenge. This model was sensitive to variation in the parameters for host resistance, survival and migration of the free-living stages of the parasites, suggesting

that these parameters are the major determinants of the observed pattern of epidemiology. Furthermore, this sensitivity implied that large reductions in pasture contamination would be required to significantly reduce worm numbers in the host, due to density dependent effects of host immunity. Therefore, they suggested that there may be more potential to influence the parasite population through selecting for increased host resistance or through vaccine development rather than manipulating the free living stages of a parasite or other control measures such as the usage of nematofagus fungi. Their model also implied that a greater impact on the pasture contamination would be gained if the egg output was reduced throughout the season rather than at the end of the grazing season. Therefore, the maximum benefit could be obtained if the efforts for breeding for disease resistance are concentrated on the earlier expression of the hosts' immunity which would lead to a reduction of the recontamination of the pasture by lambs.

Bishop and Stear (1997) described a general framework that enabled responses to selection for resistance to gastrointestinal parasites to be stochastically modeled. In their model they defined heritable, between-animal variation for pasture intake and for each major interaction between the parasite and the host, namely establishment of larvae, fecundity of the adult female parasites and parasite mortality. All the other traits (e.g. worm burden, faecal egg count etc.) were estimated as output variables from the model. Using this framework they simulated selection for reduced faecal egg count for ten years, under different management techniques. The theoretical prediction of the correlated response to selection in live-weight, ignoring the epidemiological effects, was a linear increase of the mean live-weight over the years. When the epidemiological effects were taken into account the response to selection was curvilinear as a result of exploiting environmental factors. The most effective scenario was when the animals were grazing the same field each year, taking advantage of the reduced number of larvae over-wintering from the last grazing season. The observed

responses to selection were found to be (when the epidemiological effects were exploited) up to twice those calculated by quantitative genetics theory ignoring the disease epidemiology.

Bishop and Stear (1997) found that the distribution of faecal egg count became more aggregated over time as selection progressed, where proportionally fewer animals contributed a disproportionate number of eggs to the pasture. This resulted in decreased selection differentials as selection progressed. The increase of aggregation of the distribution of faecal egg count between animals as selection progresses implies that culling of wormy animals, as a means of reducing pasture contamination, may become relatively more effective than in unselected animals.

Using as a basis the above model, Bishop and Stear (1999) explored the genetic and epidemiological relationships between productivity and resistance to gastrointestinal parasites. In the model of Bishop and Stear (1999) food intake and live-weight are not estimated on a daily basis for each animal. Instead a food intake and a live-weight (which could be achieved under no parasitism) are initially assumed. This phenotype of the animal for these two traits is comprised from a genetic, an environmental and a maternal effects. The genotypic effect is assumed to be constant whereas the environmental and maternal effects change over time. The environmental effect is assumed to be constant over a period of ten days whereas the maternal effect is reduced after three months of age. The production penalties are estimated in a cumulative manner and are subtracted from the assumed live-weight under no parasitism, at six months of age. The initially assumed correlation between food intake and live-weight is 0.5. In this study Bishop and Stear (1999) predicted a weak phenotypic correlation (mean=-0.10) between faecal egg count and observed live weight, in agreement with experimental observations. The genetic correlation between these traits was predicted to be stronger and also favourable, i.e. negative, having a mean value of -0.27. The

genetic correlation between live weight and faecal egg count was strongly influenced by the epidemiology of the disease, changing from -0.02 to -0.46 as the disease severity changed from mild to severe. The heritabilities estimated in this study were 0.17 for live weight and 0.29 for faecal egg count.

Models have proven very useful in designing breeding strategies for resistance to nematodes, as explained above, because they have provided a) an insight into the epidemiology of the disease and b) an assessment of the impact of the disease epidemiology on production traits. These studies would have been difficult to perform in a sheep flock as it would have been difficult, if not impossible, to extract all the information.

1.12 Incorporating gastrointestinal nematode resistance in breeding programmes

Breeders are cautious in taking up new and novel traits in the selection objective and selection criteria they implement, as they do not want to incur extra effort or cost for little extra return. Therefore any new trait proposed for use in a selection index should have substantial evidence backing its importance. Breeders that foresee an area towards which the market is moving, however, and take the risk of modifying their selection scheme so as to accommodate this change would have an advantage compared to their competitors. Breeding for resistance to nematodes is such an area and in this context the question that arises is: is there scope for selecting for resistance for nematode parasites?

The first and basic requirement for a trait to be included in a breeding programme is for it to be variable and heritable. Furthermore, it is desirable that it is not in a major antagonistic relationship with other traits in the selection objective. As the inclusion of the trait in the

program will slow down the improvement of the other traits, it must result in a net economic benefit.

Inclusion of resistance to parasites should be considered if the animals are going to be bred, or their offspring produce, in an environment where they will face a challenge by parasites. In general, this is the case for nematodes. In temperate regions the predominant species is *T. circumcincta* whereas in tropical regions the predominant species is *H. contortous.* As shown in a previous section the indicator trait of resistance (faecal egg count), is moderately heritable. The trait, in general, has also a low correlation with production traits (i.e. live weight). However, theoretical studies (Bishop and Stear, 1999) have shown that there are potential environmental benefits to be exploited from the inclusion of resistance into a breeding programme. Furthermore, there is evidence that in breeding programmes after several years of selection for resistance, flocks can be run without anthelminthics and have the same productivity as a flock selected for productivity and treated with anthelminthics (Bishop, personal communication). In cases like the above there is a potential marketing advantage to be exploited, since the breeder can market animals which are as productive as the competitors but additionally they are more resistant to gastrointestinal parasites. The fact that there are commercial breeding programmes in Australia (Anonymous, 1994) and in New Zealand for nematode resistance indicates that breeding for resistance to gastrointestinal nematodes could be attractive. However, we should be cautious in extrapolating from the Australian and New Zealand results, since in other countries there are different production systems, parasite species, economics of production, etc.

The major problems of incorporating resistance to nematode parasites in a breeding programme are:

- The difficulty in assigning an economic value to resistance traits.
- The uncertainty about genetic correlations between production traits and disease resistance. A further issue is that the genetic correlations may change as the disease challenge changes (Bishop and Stear, 1999).

A question also arises as to the relationship between resistance to different diseases. In other words, will selecting for one disease increase resistance to another disease? Unpublished results suggest that the genetic correlation of faecal egg count of Strongyles and faecal egg count of Nematodirus is close to 0.5 (Bishop, personal communication). Thus, selecting for resistance to parasites of one genera, should also increase the resistance to parasites of the other genera. As far as other diseases are concerned the information available is very limited. The results to date indicate that selection for resistance to gastrointestinal parasites will not have a marked effect on resistance/susceptibility to any other diseases (Raadsma *et al.*1997b, 1998).

1.13 Interaction of genetic resistance to gastrointestinal parasites and nutrition

As mentioned before it is unlikely that breeding for resistance to gastrointestinal parasites alone will be used for worm control. Therefore, the interaction between breeding and other methods should be examined, one of which is nutrition-breeding interaction. There have been several experiments exploring the effect of nutrition and several exploring the effect of genotype on the resistance to worms but very few examining the combined effect or interaction of nutrition and genotype. Results from the nutrition x genotype interaction studies will be given and a proposed theoretical framework which might be useful for understanding this interaction will be described.

Gray (1997) examined the interaction of nutrition with genetic resistance in Merino lambs. The lambs were divided into two groups: resistant and random bred which were either supplemented with protein or not, resulting thus into four groups: resistant, resistant supplemented, random bred and random bred supplemented. The supplementation was performed at 8-11 months of age and the lambs were exposed to natural and artificial infection. There was a reduction in faecal egg counts in both groups that received supplementation during the period of supplementation. The animals were then artificially challenged at thirteen months of age with 10000 infective larvae of *H. contortus* three weeks before the faecal egg count measurements were taken. After challenge, faecal egg count rose in all groups until week 5 when all lambs were drenched. During this period supplementation reduced the faecal egg count in all weeks but only in the random bred group. No differences were detected among the two resistant and the random bred supplemented groups at any stage of infection. Thus an interaction between nutrition and genotype might be inferred.

In a study involving one relatively resistant breed (Scottish Blackface) and one relatively susceptible breed (Finn Dorset), Abbot *et al.* (1985) found that supplementation with protein reduced the faecal egg count of the susceptible genotypes (Finn Dorset) but not of the resistant genotypes (Scottish Blackface). This reduction of faecal egg counts would have the effect of 'altering' the estimated correlation between faecal egg counts and production traits as the protein level increased, since the susceptible genotypes would appear to have lower faecal egg counts than if they were unsupplemented.

Coop and Kyriazakis (1999) advocated a partitioning of nutrients framework for the allocation of different nutrients to different functions. According to these authors, the function of immunity in lambs is divided into two parts: a) acquisition of immunity, and b) expression of immunity. These two processes are continuous and one does not exclude the

other, but the end of the phase of acquisition of immunity can be considered to be when: 'all the various mechanisms of the immune system start to operate'. According to their partition framework the time when the effect of the immune system on parasitic burden becomes apparent is independent of the host nutrition but it might be dependent on the parasite species. Thus, these authors maintain that host nutrition will not affect the rate of acquisition of immunity.

The second phase, namely the expression of immunity, will be affected by the hosts nutrition. The suggested prioritisation of the different body functions according to their framework is: a) maintenance of body protein, b) protein gain, c) expression of immunity, and d) maintenance and gain of body lipid. When the host has access to limited nutrients these functions will be affected in the reverse order. Therefore, animals with higher requirements for growth will express acquired immunity to a lesser extent than animals of lower requirements given access to the same amount of food, when food intake is less than full requirements. The degree of expression of immunity is expected to be affected by the relationship between stage of growth and degree of limitation in nutrient intake. As a consequence, it is unlikely that an animal which is genetically relatively resistant to parasitic challenge will benefit further by an increase in nutrient supply unless it is being maintained on a low plain of nutrition.

This partition framework must be viewed as a possible suggestion of how the nutrients will be allocated when an animal is infected by gastrointestinal parasites. The data verifying this are scanty and emphasis has to be placed on exploring the way in which animals make the best use of the nutrients they receive. It could alternatively be proposed, for example, that the ranking of functions is not universal and that resistant animals prioritise the function of expression of immunity over growth whereas susceptible animals do it the reverse way.

1.14 Summary

1.14.1 Current status of knowledge

There have been various projects in different countries investigating the quantitative genetic aspect of resistances to gastrointestinal nematodes in small ruminants. Resistance to gastrointestinal parasites (worm burden and/or mass) is not a straightforward trait to measure directly, as it would involve the slaughter of the animal. Thus an indicator trait is employed: usually faecal egg count. The main difference between quantitative genetic studies of resistance to gastrointestinal parasites and studies looking at 'conventional' productivity traits like milk yield and live-weight is that there are two living organisms involved, thus the overall impact of an animal breeding project should take into account the epidemiology of the disease.

There is a difference in the resistance levels exhibited by sheep and goats. Sheep are much more resistant to infection with gastrointestinal parasites than goats. The reason(s) for this difference are not clear although it is tempting to hypothesise that the different evolution of the feeding patterns of these two species is responsible for this difference: sheep are grazers while goats are browsers. Thus goats would, normally, be far less exposed to parasites since the faeces with the parasite eggs are shed on the ground, and hence goats would have had less pressure to evolve resistance.

Resistance (or susceptibility) to gastrointestinal parasites is not uniform among all ages and sexes of animals. Animals which are under stress (metabolic or other type) are the main susceptible classes of animals: for example lambs and periparturient ewes. Adult animals, not under stress, are quite resistant. Generally there is also a sex difference with the males being more susceptible than the females. The mechanisms leading to this difference in resistance are not fully understood.

The genetic parameter estimates show that there is potential for breeding lambs resistant to gastrointestinal parasites. Care should be taken when comparing different studies with respect to the age of the animals and the parasite species with which the animals were (predominantly) infected. In general the heritability estimates are in the region of 0.2-0.4 for lambs and 0-0.2 for goats. Perhaps this difference might be a consequence of the lower evolutionary pressure on goats for developing resistance to nematodes due to their feeding behaviour. The genetic correlation estimates between faecal egg count and productivity vary from large and negative, i.e. favourable (Bishop *et al.* 1996, Bouix *et al.* 1998), to moderately positive (McEwan *et al.* 1992, 1995). The phenotypic correlation estimates are usually close to zero and have the same sign as the genetic correlations. Reasons for the differences among the genetic correlation estimations are unknown but factors like management, parasite species, breed of sheep, etc, could affect them.

The distribution of faecal egg counts in a flock of sheep is usually skewed and, in general, often fits a negative binomial distribution, where few animals harbour the majority of the parasites. There has not, so far, been an attempt to exploit this aspect of faecal egg counts.

Despite some early conflicting results, the effect of protein supplementation on reducing the adverse effects of gastrointestinal parasitism is nowadays well established (Sykes and Coop 2001). Furthermore, a theoretical framework has been proposed for the interaction of parasites with protein nutrition. A genotype x nutrition interaction has been observed in two studies (Gray 1997, Abbot *et al.* 1985). However, there is no quantification of the impact that such an interaction could have on the estimates of genetic parameters, although it has been speculated that different protein levels in the pasture could be a factor contributing to the differences in parameter estimates.

1.14.2 Areas requiring research

Despite the fact that much is known about the quantitative genetic control of resistance to nematodes in small ruminants, several gaps still exist, in particular, in areas that may affect the implementation and effectiveness of breeding programmes for resistance. In this thesis an attempt is made to fill these gaps.

Although there have been several studies in different countries in which genetic parameters for faecal egg counts have been estimated for sheep, there have been somewhat fewer for goats. Chapter two of this thesis comprises an analyses of a data set collected from cashmere goats in Scotland, consisting of data on both faecal egg count and production traits.

Applications of new techniques for analysing repeated faecal egg count data would be useful. In recent years statistical techniques, such as random regression, have been developed and allow us to gain more information than the use of repeatability and multivariate models for datasets where we have repeated measurements on the same animal (Jaffrezic and Pletcher, 2000). Such techniques have not previously been applied to faecal egg count data. In chapter three a dataset from a Scottish sheep farm is analysed using a random regression model, in order to maximise the information extractable from the dataset.

There has not been an attempt previously to use the properties of the distribution of faecal egg count in a beneficial way. If a few animals carry a high proportion of the parasite worm burden, then separating/removing the most infected animals could be a complementary measure for increasing the production of the remaining animals by removing the major source of pasture larval contamination. In chapter four the model of Bishop and Stear (1999)

is extended so as to combine separation/culling of different percentages of the "wormiest" animals with long-term selection.

Of the proposed novel approaches for tackling the problem of gastrointestinal parasites, none has the potential of replacing anthelminthic drugs; rather they will probably be used as complementary measures. For the best results in a breeding scheme, the effect of the complementary control measures in addition to genetic selection should be examined. A strong candidate, as a complement to selection, is nutritional supplementation of the animals. So it would be very useful to know if nutritionally supplementing the animals will have an impact on the population genetic parameters. In chapter five, the effect of nutritional (and specifically protein) supplementation on the genetic and phenotypic parameters of an unselected population of lambs is explored, *in silico*.

2. Genetic control of resistance to gastro-intestinal parasites in crossbred cashmere-producing goats: responses to selection, genetic parameters and relationships with production traits

2.1 Introduction

As described in chapter one, goats and sheep are normally managed under conditions which expose them to gastrointestinal parasites, often leading to chronic subclinical infection and to loss of production by the host. Growth rate in lambs has been estimated to be reduced by up to, or even in excess of, 25% in UK conditions (Coop *et al.* 1985), where *Teladorsagia circumcincta* is the predominant parasite species.

Parasite control is normally achieved by a combination of anthelminthic treatment and pasture management. However, in recent decades there has been increasing concern about the development of anthelminthic resistance in parasite populations (Jackson and Coop, 2000). For goats, in particular, it has been demonstrated that parasites harboured by goats develop resistance more quickly to anthelminthics than parasites harboured by sheep (Jackson and Coop, 2000). Control strategies, which are complementary to the use of anthelminthics and grazing management, are sought. Selection of sheep and goats for enhanced resistance to nematode infections is such an option.

Studies investigating genetic control of the resistance of sheep and goats to nematode parasites are summarised in chapter one. It is apparent that there have been fewer studies on goats than sheep and current knowledge is not as comprehensive. There is evidence that goats are more susceptible than sheep to gastrointestinal nematode parasites (Le Jambre, 1984; Pomroy *et al.,* 1986; Huntley *et al.*, 1995). Therefore, it might be expected that the reduction in productivity and financial losses might be higher in goats than in sheep. However, in contrast with sheep, the heritabilities for faecal egg counts in goats are typically

low (Baker *et al.* 2001, Woolaston *et al.*, 1992, Morris *et al.*, 1997b, Mandonnet *et al.*, 2001).

As outlined in Chapter 1 (Section 1.11 and 1.12), effective inclusion of resistance to gastrointestinal parasites into breeding schemes should be beneficial – improving resistance will help to alleviate concerns over treatment strategies and may also help to improve productivity. However, effective inclusion of resistance traits into breeding schemes requires knowledge of the genetic relationships between resistance and productivity. These relationships in sheep are currently contentious, because no clear pattern has emerged from research undertaken on Scottish Blackface sheep under upland conditions, Australian Merinos or New Zealand dual-purpose sheep. For goats there have been fewer studies and therefore the information available is much more limited. As goats are more susceptible to parasites and the parasites harboured by goats develop resistance to anthelminthics more quickly than the parasites harboured by sheep, the option of selecting goats resistant to gastrointestinal parasites needs to be explored and estimates of the heritability of resistance and the relationship between resistance and production traits need to be investigated.

To investigate the potential of breeding cashmere-producing goats for resistance to gastrointestinal nematode parasites, a study was initiated in Scotland in 1992 in which goats were selected for increased resistance, using faecal egg counts as the indicator trait. The study was prompted by the development of multiple anthelminthic resistance in the herd (Jackson *et al.* 1992). The aims of this chapter are threefold: firstly to estimate responses to selection for increased resistance to gastrointestinal parasites, secondly to estimate genetic and phenotypic parameters for faecal egg counts and thirdly to estimate correlations with production traits. The production traits considered were live-weight and traits related to fibre production and quality.

2.2 Materials and Methods

2.2.1 Goat population

The population studied has been described previously by Bishop and Russel (1994 and 1996). The goats were derived from Scottish feral goats and importation of animals, embryos and semen from Iceland, Tasmania, New Zealand and the Gorno Altai region of Siberia, made between 1986 and 1988. A crossbreeding programme amongst the strains of goats produced a variety of purebred, two and three way crosses, distributed across a total of 18 commercial farms, including the Macaulay Institute's Sourhope Research Station, which served as a nucleus herd. The does gave birth to their first kids when they were approximately two years of age and were retained for 3 to 5 parities. The number of kids evaluated each year for production traits (see below) and included in the dataset is shown in Table 2.1, as are the number of sires and dams contributing to this dataset. From 1992 onwards only kids born in the nucleus herd were evaluated.

Selection, as described below, was initiated for fibre traits in 1991 and nematode resistance in 1992. The nucleus was initially divided into three lines: a line selected on an index to improve the value of cashmere produced (190 does), a line selected for decreased fibre diameter (95 does) and an unselected control line (70 does). In 1992 another line was created, selected for resistance to gastrointestinal nematodes (helminth line, 95 does).

Table 2.1 Number of goats with production trait measurements

Year of birth	Kids	Sires	Dams
1987	99	4	55
1988	110	11	74
1989	100	19	58
1990	275	26	168
1991	239	44	162
1992	358	15	311
1993	388	16	336
1994	374	16	316
1995	403	15	371
1996	299	17	263
1997	455	15	404

2.2.2 Parasitological measurements and selection procedure

The measurement criterion to assess the degree of parasite infection was faecal egg count, i.e. the number of parasite eggs per gram of faeces. The method used for determining faecal egg counts was a saturated salt flotation technique, modified from the method described by Christie and Jackson (1982). Apart from the initial screening study described below, faecal egg counts were measured on goats of both sexes during their second grazing season when they were 12 –18 months old, as previous experimental evidence suggested that kids in their first grazing season showed little resistance to parasites, and hence received suppressive anthelminthic treatment. The number of faecal egg count measurements taken from an individual varied between years, from four to eleven.

One hundred mixed-age, adult male goats from several herds were used in an initial screening study in 1992 in which they were exposed to both artificial and natural *T. circumcincta* challenge. At the end of the challenge the two males with the lowest geometric mean faecal egg count were bred with 95 unselected does to create the helminth line. The following year the same two males plus an additional two males with low faecal egg counts were used as sires (Jackson *et al.* 1995). In subsequent years three males were selected each year from within the selection line, again using the adjusted geometric mean faecal egg count

as the selection criterion. To reduce potential inbreeding, selection was practised within family, i.e. the first cohort of selected bucks each year did not include full or half- sibs. Female replacements were chosen at random.

Responses in the helminth line were compared with goats that were unselected for nematode parasite resistance (control line). These goats generally corresponded to those used as the control for the fibre lines, but some additional goats from the value and fine lines were included to increase numbers. Selection line and control goats of the same sex were grazed together and thus faced the same potential parasite challenge. Additionally, in 1996 a single faecal egg count measurement was collected from all yearling goats on the farm not participating in the selection line *vs.* control comparison. The structure of the population with parasitological measurements is given in Table 2.2.

Table 2.2 Number of sires, kids† with faecal egg counts and number of faecal egg counts

Year of birth	Number of Kids		Number of Sires		Number of
	Selected	Control	Selected	Control	Measurements
1993	57	61	2	14	675
1994	74	76	4	11	984
1995	74	76	3	9	1034
1996	69	50	3	13	644
1997	75	83	3	6	877

† an additional 135 kids in 1996 that were not in the selection or 'control' lines had a single faecal egg count

The male yearlings were treated with anthelminthic at each sampling time for the first two years, but from year three were treated only at turnout onto pasture in spring following an exposure to artificial challenge during winter housing. The female yearlings were always challenged during winter housing and then treated at turnout onto pasture, and thereafter treated on each sampling occasion. Goats in this study seldom showed clinical signs of

nematode infection. Faecal egg counts from the artificial challenge are not included in this analysis.

2.2.3 Production traits

Kids were evaluated for fibre characteristics on the basis of a 10 cm^2 mid-side patch of fleece sample taken at about 5 months of age. The mid-size sampling site was over the last rib halfway up the side of the animal, as this site is considered to be representative of the fleece as a whole (Pattie *et al.* 1989). Each year kids were sampled and recorded from all available lines and the selection procedure was based on adjusted phenotypic records.

The traits measured were as follows: liveweight at five months of age (LW), cashmere weight in the 10 cm^2 patch sample (P_Cash), mean cashmere fibre diameter (Diam) and the standard deviation of fibre diameters in each sample (Diam_sd) and cashmere yield (Yield). Yield is cashmere weight as a proportion of total fibre weight. Until 1996, fibre diameter was measured following manual separation and weighing of guard hair and cashmere fibres, by projection microscopy using standard procedures (International Wool Textile Organisation, 1989). In 1997, fibre diameter was determined by optical fibre diameter analyser (OFDA) according to International Wool Textile Organisation Specification IWTO-47-95. In the first two years of the trial, fibre length was measured directly on the animal at the time of sampling, however this measurement was subsequently discontinued. From 1992 onwards, staple length was measured on the laboratory samples, and for the purpose of analyses this is treated as the same trait as fibre length. Live weight was also recorded at the time of fibre sampling.

2.2.4 Data analysis

Distributions. The faecal egg count (Fec) measurements and cashmere traits were generally not normally distributed. It has been reported many times that Fec measurements often fit the negative binomial distribution. This distribution describes traits which show overdispersion in comparison with a normal or even a poisson distribution, with a small number of animals contributing a large proportion of the larvae to the pasture (Barger 1985, Stear *et al.* 1995, Stear *et al.* 1998). For example, in New Zealand studies it has been estimated that the most susceptible 10 % of animals contribute 50% of the pasture contamination (McEwan, 1994). The negative binomial distribution is described by two parameters: m, the mean and k which describes the aggregation of the distribution: as $k \rightarrow 0$ the majority of the parasite population is concentrated on fewer animals, and as $k \rightarrow \infty$ the parasite population is more randomly distributed (Anderson and May, 1992). In this dataset the negative binomial distribution was fitted to the faecal egg count dataset within year, sex and line using the GENSTAT package (Lawes Agricultural Trust, 1993). GENSTAT tests the fit of the data to the specified distribution using a χ^2 test of association.

Two methods were used to identify the best transformation that would render faecal egg counts approximately normally distributed. Firstly, commonly used transformations were made to the trait (natural logarithm, square and cubic root) and they were tested for normality using the GENSTAT package (Lawes Agricultural Trust, 1993). This algorithm uses a χ^2 test of association for testing the fit of the data with the defined distribution. The second method was Box-Cox transformation using the equation given by Sokal and Rohlf (1995). The objective of this method is to find the best transformation for a given dataset (Y). This is done by estimating $Y' = (Y^{\lambda} - 1)/\lambda$, for $\lambda \neq 0$, and $Y' = \ln Y$, for $\lambda = 0$. A log likelihood is then estimated as $L = -\frac{\nu}{2}\ln s_T^{\,2} + (\lambda - 1)\frac{\nu}{n}\sum \ln y$, where, ν are degrees of

freedom from ANOVA and s_T^2 is the error mean square based on n degrees of freedom. The value of λ which maximises the log-likelihood function yields the best transformation to normality. The best transformation (from both methods) was found to be the cubic root (see results section).

The production traits were tested for normality using the GENSTAT statistical package (Lawes Agricultural Trust, 1993). P_Cash was found to be the only cashmere trait not approximately normally distributed and therefore was analysed both in the logarithmic scale and in the observed scale. Fibre diameter was also additionally analysed on the logarithmic scale because P_Cash is multiplicative or a power function of diameter. Considering these traits on the logarithmic scale will convert these to linear relationships (Bishop and Russel, 1996). Subsequent analyses were performed on both the transformed and the untransformed data.

Statistical model specification. Least squares analysis of variance was undertaken in order to identify important sources of variation. Fixed effects which were fitted included year, farm, line, date of birth, sex, if the animal was born to its genetic mother or by MOET procedure (practised to spread genotypes across farms during the initial expansion of the population) and litter size. The genetic origin of the animals i.e. proportion of different genotypes, was also fitted. For the Fec the proportion of Icelandic genotype was the only genetic group effect found to be significant and it was fitted as a covariate [regression coefficient $\sqrt[3]{\text{Fec}}$ = 1.70 (s.e. 0.49)]. All the significant first-degree interactions were fitted as well.

The same fixed effects described above were used for analyses of production traits, and the same procedure for finding the appropriate model was performed. For fibre diameter a code

for the operator who performed the measurement was also available and fitted as a fixed effect.

The fixed effects which were found to be significant were fitted in a REML analysis performed in GENSTAT (Lawes Agricultural Trust, 1993), fitting also sire as a random effect. For each year the mean faecal egg counts of the selected line and control animals were compared using a t test.

Genetic parameter estimation An animal model was fitted, using ASREML (Gilmour *et al.* 1996), for estimating the additive genetic variance components for both the faecal egg count measurements and the production traits, using all known pedigree relationships. For faecal egg counts ($\sqrt[3]{\text{Fec}}$), permanent environmental effects, i.e. the environment covariance between the repeated observations on the same animal, and genetic maternal effects were fitted in turn. The resulting estimates of heritability are estimates of a single Fec measurements. It should be noted that 'single' Fec measurement is used to denote the inferred properties of a single observation from repeated record analysis of a dataset where all available records per animal were used. A model which included maternal genetic effects or litter effect, both with and without a covariance between the maternal and the additive genetic component, was tested for all the traits. The likelihood ratio test was used to distinguish between models and select the most parsimonious model.

The genetic strain of the goat has a large effect on all production traits (Bishop and Russel, 1994, 1996). It was accounted for by regressing the measurement for each kid on the proportion of each of the five strains which comprised its own genotype. This assumes no heterosis effects, which is a reasonable assumption for the fibre traits (Bishop and Russel,

1994). The resulting heritabilities may be thought of as within-strain heritabilities. For Fec, as described above, only the proportion of Icelandic genotype was fitted as a covariate.

Bivariate analyses of the production traits with faecal egg counts were performed using the mean of the faecal egg counts measurements for each animal for estimating genetic and phenotypic correlations between traits. All available production data were used to maximise the precision of the estimates. The effects that were fitted for each trait were the same as those fitted at the univariate analyses of the trait. Phenotypic correlations for a single faecal egg count measurement were estimated from the results, rescaling the environmental variance by the average number of measurements per animal.

2.3 Results

2.3.1 Summary of traits and their distribution

In Table 2.3 the mean, minimum and maximum values for each trait are shown along with the phenotypic standard deviations.

Table 2.3 Summary statistics for each trait

Trait	Units	Min	Mean	Max	σ_p
Faecal egg count	eggs/g	0	268	4410	
Cubic root (Faecal egg counts+1)	$\sqrt[3]{eggs/g}$	1	6.62	16.4	1.36
Live weight at 5 months of age	kg	8.5	16.8	34.5	3.46
Length of fibres	mm	10	46.9	80	9.97
Cashmere weight in the 10 cm2 patch sample (P_Cash)	g	0.06	0.26	1.34	0.12
Yield	100xg/g	3.9	48.2	86.5	12.3
Diameter (Diam)	µm	11.1	15.1	24.7	1.42
Diameter standard deviation (Diam_sd)	µm	1	2.89	6.5	0.57

For faecal egg counts, the cubic root transformation proved better than the square root or the logarithmic transformation in making the data approximately normally distributed. Box-Cox transformation gave a λ value of 0.33 indicating that the cubic root was an appropriate transformation for this particular dataset. It should be noted that the interpretation of λ is empirical, however there are particular values which point to commonly used transformations: 1 points to the linear, 0 to the logarithmic, 0.5 to the square root and 0.3 to the cubic root. For the production traits, P_Cash was skewed and not normally distributed, but a logarithmic transformation was used to create an approximately normally distributed trait.

The negative binomial distribution was fitted to the faecal egg count measurements. The data fitted the negative binomial distribution only in four out of twenty year x line x sex cohorts. The parameter k showed that for the cases where the negative binomial distribution did not fit the data due to a lack of aggregation of the data.

Averaging across the whole dataset, the parasite burden of the 5% of the animals with the highest Fec contributed 19% of eggs to the pasture and the parasite burden of the 10 % animals with the highest Fec contributed 31% of the eggs to the pasture.

2.3.2 Selection responses for faecal egg counts

In Figure 2.1 the mean of the cubic root transformed faecal egg counts (CFEC) of both the control and the selected animals are given, along with the standard errors of the mean. The mean faecal egg count values are not shown for the pre-screening population of the sires as there is no valid comparison between those and subsequent values. Therefore, the results are given from the first generation of selected animals onwards.

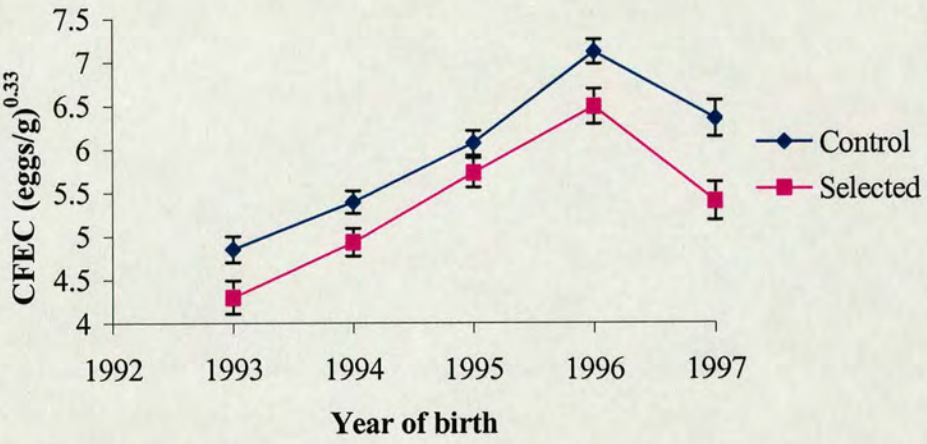

Figure 2.1 Mean of CFEC ± 1 s.e. for both the selected and the control line

In Figure 2.2 the back-transformed means are shown for the cubic root transformed faecal egg count, for both lines.

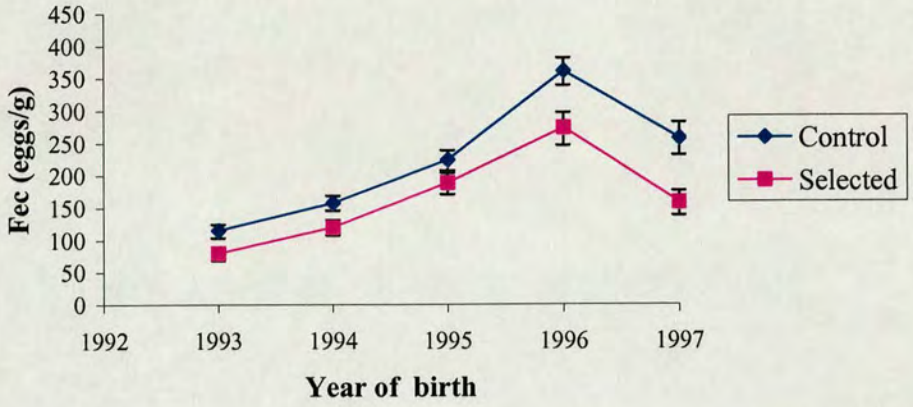

Figure 2.2 Back-transformed means for both selected and control lines, after analysis on cubic root scale. Error bars = $(\sqrt[3]{(\bar{x}+1)} \pm \text{s.e})^3$

2.3.3 Heritabilities of faecal egg count

For Fec the models which included maternal genetic effects or permanent environmental effects were found to fit the data no better than the ones without them, using the likelihood ratio test. However, fitting the permanent environmental effects allowed estimation of the within-season repeatability of faecal egg count. In Table 2.4 heritabilities and repeatabilities and their standard errors are given for faecal egg count, on the cubic root scale for a single faecal egg count measurement (CFEC). The heritability of the mean faecal egg count for each animal was 0.32 (s.e. 0.09). These parameters were estimated for the case when the proportion of Icelandic genotype was fitted as a covariate. When the mean of repeated measurements was taken the heritability on the cubic root scale increased to 0.39 (s.e. 0.08).

Table 2.4 Heritabilities (h^2) and repeatability (r_{ep}) of Fec on the cubic root scale.

CFEC	$h^2_{\dagger}$	s.e.	$h^2_{\ddagger}$	s.e.	r_{ep}	s.e.
% Icelandic Fitted	0.17	0.02	0.11	0.03	0.17	0.02
% Icelandic not Fitted	0.18	0.02	0.14	0.03	0.18	0.02

† h^2: permanent environmental effects not fitted
‡ h^2: permanent environmental effects fitted

2.3.4 Heritabilities of production traits

Heritabilities with standard errors are given in Table 2.5 for the cashmere traits on both the logarithmic and observed scale. It should be noted that the heritabilities of live weight were estimated with a model that also included maternal genetic effects. Fitting the covariance between direct genetic and genetic maternal effects did not improve the model and therefore the covariance of these effects was not included in the analysis. The maternal genetic effect for live weight, as a proportion of phenotypic variance, was estimated to be 0.28 (s.e 0.03). When the maternal genetic effects were not included the heritability estimate was 0.50 (s.e. 0.05).

Table 2. 5 Heritabilities (and s.e.) of cashmere traits

Trait	h^2	s.e.
Length	0.57	0.05
P_Cash	0.49	0.04
Yield	0.65	0.04
Diam	0.64	0.04
Diam_sd	0.28	0.04
Log(P_Cash)	0.61	0.04
Log(Diam)	0.63	0.04
Live weight	0.22	0.05

2.3.5 Correlations between faecal egg counts and production traits

In Table 2.6 the genetic, phenotypic and environmental correlations of production traits with the mean of several faecal egg count measurements and with one measurement are shown. Standard errors are shown for genetic correlations. As can be seen, all the correlations are

small to moderate, and not significantly different from zero (as judged by their standard errors), although the genetic correlations are always more positive than the phenotypic and the environmental. The environmental correlations are all slightly negative.

Table 2.6 Genetic (r_g), phenotypic (r_p) and environmental(r_e) correlations of Fec (cubic scale) with production traits, both for mean of several Fec measurements and for single measurements

Trait	CFEC			
	r_g	s.e.	r_p†	r_e†
Length	0.23	0.15	0.06	-0.08
			0.04	-0.04
P_Cash	0.10	0.15	-0.03	-0.10
			-0.02	-0.06
Yield	0.16	0.13	0.05	-0.05
			0.03	-0.03
Diam	0.19	0.16	-0.01	-0.14
			-0.00	-0.08
Diam_sd	0.30	0.19	0.07	-0.02
			0.05	-0.01
Log(P_Cash)	0.10	0.14	-0.02	-0.11
			-0.01	-0.06
Log(Diam)	0.21	0.16	0.03	-0.08
			0.00	-0.05
Liveweight	0.00	0.15	-0.01	-0.03
			-0.01	-0.01

† The first line for each trait is the correlation with the mean Fec and the second is the deduced correlation for a single Fec measurement

2.4 Discussion

The aim of this study has been to explore the possibility of breeding for resistance to gastrointestinal parasites in goats. The indicator trait of resistance to gastrointestinal parasites, faecal egg count, was found to be heritable albeit less so than in sheep. The fibre related traits were found to be generally highly heritable. Live weight was the only trait for which maternal effects were found to be important and after accounting for these effects this trait was found to be moderately heritable. The genetic correlations between production traits and faecal egg count were always positive, although not significantly different from zero, whereas the phenotypic and environmental correlations were clustered around zero.

Many previous experiments in sheep have shown that it is possible to breed for reduced faecal egg counts. The mean faecal egg count of the control and the selected lines in this study were different in the first three years and there was a tendency in the last two years for the two lines to diverge further apart. The fact that the divergence of the two lines was more evident in the last two years could be due to a number of factors such as random genetic drift, the absence of selection in females and the relatively small number of available animals. The significant difference of the two lines in conjunction with the fact that faecal egg count is a heritable trait shows that successful breeding for reduced faecal egg counts is possible not only in sheep but also in goats.

The heritability of a single Fec measurement was found to be low compared to the majority of values published for sheep (usual range 0.2-0.4). The heritability of a single Fec measurement when permanent environmental effects were not fitted was found to be equal to the repeatability when permanent environmental effects were fitted. There are indications from this dataset that the environmental correlation changes between different measurements. Measurements taken close together in time tend to have positive correlation

but the correlation reduces, even becoming negative, as the time between measurements increases.

Previous studies have suggested that the heritability of Fec in goats is relatively low. Woolaston *et al.* (1992) estimated a heritability of 0.04 (0.03) for weaners and 0.08 (0.06) for adults. The experiment was conducted in Fiji and involved 1513 weaners (defined as <365 days old) and 789 adult goats (defined as >365 days old). The permanent environmental effect was 0.00 (0.11) which indicated that the repeatability is not greater than the heritability. Morris *et al.* (1997b) in a study of Saanen does in New Zealand estimated an overall heritability of faecal egg counts of 0.05 (0.03). It must be noted that all the other studies have been performed in the tropics where *Haemonchus contortus* is the predominant species. This study was performed in a temperate region (Scotland) where the predominant species is *T. circumcincta*.

Heritability estimates presented by Baker (1998) for goats under African conditions were 0.11 (s.e. 0.11) for weaners and 0.03 (s.e. 0.03) for kids at 6 months of age. Assuming that the same, or similar, immunological mechanisms act in both goats and sheep, and given the observation (Bishop *et al.* 1996) that the expression of genetic control in sheep increases with age, the kids studied by Baker (1998) may have been too young for their defence mechanisms to be effective. In a subsequent analysis of the same dataset, Baker *et al.* (2001) estimated heritabilities of 0.08 (s.e. 0.07) at two months of age, 0.26 (s.e. 0.15) at four and a half months of age and 0.08 (s.e. 0.12) at fourteen months of age, although rather small number of animals were measured.

Mandonnet *et al.* (2001), in a study in Guadeloupe in the French West Indies, estimated genetic parameters for resistance to worms in a population of Creole goats. Their estimate of

heritability was 0.20 at 82 days of age, 0.14 (s.e. 0.05) at four months of age and 0.33 (s.e. 0.06) at ten months of age. The heritabilities at the four and ten months of age were estimated for the mean of two measurements taken one week apart. Assuming that they represent the same infection these heritabilities are higher than those which would have been estimated on the basis of one measurement.

Naïve lambs start to mount an immune response, demonstrated by an increase to the heritability of the trait, when they come to be challenged by parasites. Faecal egg counts appear to become a heritable trait for lambs at approximately three months of age (Bishop *et al* 1996). Lloyd (1987) suggested that information from sheep population studies could be used to extrapolate for goat populations. It could be suggested that goats develop an immunity response in a pattern similar to sheep. However, goats are more susceptible to parasites than sheep (Le Jambre 1984, Pomroy *et al.* 1986, Huntley *et al.* 1995), although the reasons for this increased susceptibility are not known. It may be hypothesised that the feeding behaviour of goats (browsing), which would result in exposure to lower levels of larval intake than in sheep (grazing), has led this species to a different evolutionary pathway where resistance to parasites was (is) not an important trait because goats were (are) not exposed to such high levels of parasites as sheep. This increased susceptibility might also be accompanied by a late expression of immunity. Therefore goats might express a genetically influenced immune response at a later age than sheep (Baker, personal communication). The trend in the estimates of Baker *et al.* (2001) is an increase of the heritability during the first four and a half months of an animals life, then a phase where the heritability does not vary much until ten months of age and then decreases until the fourteenth month of age (last data point). Given this observation it could be suggested that the increase in heritability is associated with a phase of acquisition of immunity, which may last longer in goats compared

with sheep. However, as mentioned before the standard errors of the estimates of Baker *et al.* (2001) were large and the estimates themselves were not significantly different.

As mentioned above, the heritability of the mean of several measurements is higher than that of a single measurement, as taking the mean of multiple measurements reduces the variance due to the reduction of non-permanent environmental effects. The amount by which it is reduced depends on the number of measurements taken and the repeatability. For a trait such as faecal egg counts for which the environmental conditions have a large effect there is, theoretically, a lot to be gained from multiple measurements (Falconer and Mackay, 1997). In the present study the number of measurements taken was approximately 7 in 1993 and 1995, 11 in 1994, 4 in 1996 (some animals had one measurement in 1996), and 5 in 1997. The repeatability of faecal egg counts was found to be 0.17. As with all low heritability and low repeatability traits, more faecal egg count measurements will result in an increase in the heritability of the trait and therefore an increase in the accuracy of the estimate of an individual animal's breeding value. In this particular case (h^2=0.11, r_{ep}=0.17, repeatability model), using quantitative genetics theory, it can be estimated that the heritability of the mean of three measurements will increase to 0.27 and that of five measurements to 0.36 approximately. In practice, the benefit of a more accurate estimation of an individual's breeding value should be weighed against the cost of the extra measurements.

Most of the heritabilities found for cashmere traits are high compared to the estimates for other production traits, and are in general agreement with previous studies (Bishop and Russel, 1996, Gifford *et al.* 1990, Bigham *et al.* 1993). Live weight was the only trait for which significant maternal effects were found. Fitting maternal effects resulted in a change of the heritability estimate from 0.50 to 0.22. The estimated heritability was similar to that of

Gifford *et al.* (1990) and somewhat lower than that of Bishop and Russel (1996). As pointed out by Bishop and Russel (1994), live weight, unlike the fibre traits, showed heterosis. In this analysis it was assumed that there was no heterosis. The present dataset is larger than that used by Bishop and Russel (1996), i.e. it is the same dataset augmented by several more years of data. Therefore there is much more information and a deeper pedigree, which is essential for estimating maternal effects. Therefore, the difference in the estimation of the heritabilities and maternal effects are probably due to the extra information available in this analysis.

The results of the bivariate analysis indicate that faecal egg counts and cashmere traits are uncorrelated phenotypically, although there are indications of small unfavourable genetic correlations. This is in agreement with most findings for sheep, where wool production traits have a small or zero correlation with faecal egg count (Morris *et al.,* 1997a, 2000; Greef and Carlson, 1999; Woolaston, 1990). Resistance to gastrointestinal parasites in kids, as results from studies have so far shown (Baker, Personal Communication; Mandonnet *et al.,* 2001), is not genetically correlated with live weight. Therefore, selection for resistance is unlikely to have a correlated genetic effect on live weight, although it might influence fibre traits decreasing both fibre weight and diameter. Economically, however these two effects on cashmere are in the opposite direction, and predicted responses in overall fleece value using the cashmere production index, as described by Bishop and Russel (1994), are negligible. An environmental advantage of selection for reduced faecal egg counts will be lowered pasture contamination, with possible longer-term benefits in reduced parasite challenge as proposed by Bishop and Stear (1997) and refined by Leathwick *et al.* (2002).

The results of this study show that there is potential for breeding goats for reduced faecal egg counts as an indicator of resistance to gastrointestinal parasites. It is important to note that

with the currently available information, and if the hypotheses described above are true, it is more difficult to exploit the heritable variation in goats than in sheep, as they express genetic variation in resistance to gastrointestinal nematode parasites at an older age. Breeding for resistance to worms is likely to be beneficial in cases where the animals are kept for more than one year of productive life, such as in the case of milk production or fibre production, assuming that genetic differences in resistance are expressed in adult animals. As in the case of sheep there will be a benefit of multiple measurements and a compromise should be made between cost and the increases of the estimate of heritability and the accuracy of selection, e.g. h in the case of selection on individual phenotype. An aspect which needs to be addressed in studies investigating the genetic control of resistance in goats is the age at which the resistant animals express their superiority. Ward *et al.* (1999) has showed that resistant lambs begin to express resistance earlier than other lines. In goats where resistance is expressed at comparatively late ages, this is of considerable importance.

3. Estimation of heritabilities and correlations between repeated faecal egg count measurements in lambs facing natural challenge, using a random regression model.

3.1 Introduction

As described in Chapters 1 and 2 there is good evidence in sheep and goats that selection to address the important problem of nematode infection could contribute to the improvement of flock health and performance. For achieving this, a measurable trait of the host has to be used and such a trait for measuring resistance to gastrointestinal nematodes is usually faecal egg count. However, there are many technical questions to be addressed. In a commercial animal-breeding scheme we would like to take the fewest possible faecal egg count measurements so as to keep the cost low, or achieve a compromise between the cost and the accuracy of the measurements. At the same time these measurements should accurately reflect the resistance status of the host. Additionally, one would like to be confident that the measurement(s) have a reasonably high correlation with measurements that could have been taken at different time points. This is especially the case with traits which are extremely variable both across and within time, such as faecal egg count. Detailed analyses of repeated faecal egg count measurements may allow these issues to be addressed, for example by quantifying the change in heritability with age and assessing inter-age genetic correlations.

In animal breeding there are two commonly used techniques for analysing repeated measurements: either assume a genetic correlation of one between measurements taken at different time points and analyse them with a so-called repeatability model, or treat the different measurements as different traits and use a multivariate model. Neither of these two methods allows phenotypic interpolation between time points from the data and the multivariate analysis also does not allow genetic interpolation. Random regression and covariance functions are statistical techniques for analysing repeated measurements and they

are of interest for several reasons. These are described by Meyer and Hill (1997) and include a) the fact that they can produce a description at every point along the continuous (time) scale of measurement enabling an interpolation between the ages for which records are available and, b) they are likely to make more efficient use of the data. In this particular case we are mainly interested in random regression for the first reason, the fact that we can interpolate between the measurement times so as to estimate the best sampling time. Random regression models have been used for the analyses of various traits in animal breeding (see discussion of this chapter for examples).

In this chapter a data-set of faecal egg counts previously analysed by Bishop *et al.* (1996), augmented by one year's extra data, is analysed using a random regression model. The aim is to get a good description of the genetic properties of the data and how they change over time. This information can then be used to find the best sampling time for faecal egg count, within the available age range. As a result of using random regression, the change of genetic and phenotypic parameters over time for faecal egg count will be described.

3.2 Materials and Methods

3.2.1 Animals and experimental design

A description of the data collected for the first three out of the four years in this dataset was given by Bishop *et al.* (1996). The data were collected from a commercial flock of Scottish Blackface sheep on an upland farm in Scotland which were exposed to natural, mixed, predominately *T. circumcincta* infection while grazing. A total of 193, 188, 195 and 156 lambs were studied in 1992, 1993, 1994 and 1995, respectively. The lambs were sired by a total of 30 rams. Most of the lambs were twin born within a two week period. Lambs were kept in two separate fields prior to weaning each year. At about sixteen weeks of age, all lambs were moved to one field so as to minimize variation in exposure to infective larvae.

Each year faecal samples were collected from the rectum when lambs were four weeks of age on average, and thereafter at four weeks intervals until the lambs were twenty four weeks of age (twenty six weeks in 1992 and 1993) giving six samples per animal. Faecal egg counts were made from a 3g sample of faeces using the modified McMaster technique (Gordon and Whitlock, 1939; Bairden 1991) with each egg count representing fifty eggs/g. In 1993 duplicate aliquots from the same faecal sample were counted for the 4^{th}, 5^{th} and 6^{th} sampling time. In 1994 quadruplicate counts were made for faecal sample 6. In 1995 and 1996 quadruplicate counts were made for all six sampling times. The majority of larvae recovered from culture were *T. circumcincta*. Other parasites identified from the faecal samples but not analyzed were from the genera *Strongyloides*, *Nematodirus* and *Eimeria*. After collection of each faecal sample, all lambs were treated with a broad spectrum anthelminthic, which was given at the dose per kilo live-weight recommended by the manufacturer, based on the weight of the heaviest lamb at the time of the treatment. The efficacy of the anthelminthic was tested with a faecal egg count reduction test and there was no evidence of drug resistance within the flock.

3.2.3 Data analyses

The genetic analysis was completed using ASREML (Gilmour *et al.* 1996). The fixed effects fitted to the model were the same as those fitted by Bishop *et al.* (1996). They included sex, birth type, testing time and the interaction of field and year of birth and date of birth was fitted as a covariate. Date of birth accounts for the fact that the lambs were born over a time period of approximately a month. All faecal egg counts (Fec) were positively skewed and were transformed by ln(Fec+25) prior to analyses. All known genetic relationships between animals were included in the analyses.

Following initial random regression analyses (described below), problems of convergence were identified. Given the fact that the heritability of the first sampling time was found to be very low (Bishop *et al.* 1996) the data for this sampling time were excluded from further analyses, and this resolved apparent convergence problems.

Four random effects were fitted: genetic, individual animal environmental, litter and residual environmental effects. For each animal the genetic trend in Fec over time was fitted as a polynomial with random coefficients (coefficients for individual animals were fitted as deviations from a mean curve). Covariances between parameters (intercepts or slopes) of different animals were assumed proportional to the corresponding relationship elements of the **A** matrix. Hence, this polynomial represented the genetic effects. Secondly, a similar effect was fitted as a polynomial for environmental effects, namely the individual animal environmental effect. Having five measurements available, the polynomials fitted could theoretically be up to quartic, for each of the above two random effects. Initially, a polynomial of first degree was fitted for both effects. Subsequently, the degree of the polynomial was increased for one effect while the other remained of first degree. It was not

possible to fit higher than linear polynomials to both effects simultaneously, due to convergence problems. Thus at all times either the genetic or the individual animal environmental effect was fitted as polynomial of first degree with the other varying to up to third degree polynomial. A litter effect was fitted as well, constant across all time points. Attempts were made to fit a separate litter effect for each sampling time but there were convergence problems and thus it was decided to keep it constant. Finally, a random residual environmental term, specific for each of the testing times, was also fitted, describing variation between replicated measurements at a specific time point. A model with all the fixed effects described, a first degree genetic animal effect, cubic individual animal environmental effect, constant litter effect, and residual effect fitted independently for each sampling was finally fitted. This model was chosen after testing it against other models using the likelihood i.e. χ^2 test $2(\log L_1 - \log L_2)$.

The output of ASREML for each polynomial fitted included a matrix containing the values of the polynomials, Φ, and a symmetric matrix, $\hat{\mathbf{C}}_x$, containing the variances and covariances of the polynomial coefficients which was constructed following the notation and procedures of Kirkpatrick *et al.* (1990). This procedure was done both for the genetic and the individual animal environmental effect (co)variance matrix. The genetic and individual animal by test environmental (co)variance matrices were estimated as :

$$\hat{\mathbf{G}} = \Phi_1 \hat{\mathbf{C}}_G \Phi_1' \qquad (1)$$

and

$$\hat{\mathbf{E}} = \Phi_2 \hat{\mathbf{C}}_E \Phi_2' \qquad (2)$$

respectively.

The phenotypic (co)variance matrix was estimated as the sum of all variances at time *t*:

$$\hat{\mathbf{P}} = \hat{\mathbf{G}} + \hat{\mathbf{E}} + \hat{\mathbf{M}} + \hat{\mathbf{e}}$$

where $\hat{\mathbf{M}}$ is the litter effect variance matrix and $\hat{\mathbf{e}}$ the residual environmental variance matrix (diagonal, with the estimated residual environmental variances on the diagonal). Having obtained all the relevant (co)variance matrices the heritability and the genetic and phenotypic correlations between measurements taken at different times were estimated.

For interpolation the following methodology was implemented using GENSTAT (Lawes Agricultural Trust, 1993). The matrix $\mathbf{\Phi}$ was expanded adding the relevant number of rows, corresponding to the time points interpolated. For the first column the values added to these rows were a constant. For the rest of the columns these values were estimated from the relevant equations given by Kirkpatrick *et al.* (1990) for the coefficients of the polynomials. In this way the $\mathbf{\Phi}_\chi$ matrix was expanded to $\mathbf{\Phi}_{\chi^*}$, which has the same number of columns but more rows than $\mathbf{\Phi}_\chi$. In equations (1) and (2), substituting $\mathbf{\Phi}_{\chi^*}$ for $\mathbf{\Phi}_\chi$ and $\mathbf{\Phi}_{\chi^*}'$ for $\mathbf{\Phi}_\chi'$, yields the new genetic and environmental matrices. The litter (maternal) effect variance matrix, $\hat{\mathbf{M}}$, was expanded by adding an appropriate number of columns and rows with the (constant) litter variance on the diagonal and zeros in the off diagonal positions. Matrix $\hat{\mathbf{e}}$ was expanded by taking the weighted average of the appropriate variances, as the residual variance was assumed to change in a linear fashion between two estimated variances. As previously, having estimated all the relevant (co)variance matrices, the phenotypic and genetic correlation matrices were estimated along with the heritabilities for every time point.

Three time points were interpolated between different sampling times, i.e. weekly time points, giving in total seventeen time points after interpolation compared with the original five data sampling times. From these expanded matrices, the genetic and phenotypic

correlation matrices describing relationships between time points were estimated along with the heritabilities for each of these time points.

As a reference and for comparison, five univariate analyses using a repeatability model within time were performed, using ASREML, for each time point for which data were available. In these analyses, the same fixed effects were fitted as above, along with the random terms describing the genetic, litter, individual animal and residual effects. It should be noted that multivariate analyses of all the measurements was attempted but there were problems of convergence.

3.3 Results

In Table 3.1 the means and the maxima are shown for the trait analysed, ln(Fec+25), and the untransformed data. In the same table the phenotypic standard deviations for log-transformed faecal egg count, estimated from the univariate analyses, are shown. It is worth noting that the phenotypic standard deviations on the logarithmic scale in Table 3.1 are all close to unity, i.e. typical of log-transformed faecal egg count. The distribution of the measurements before the transformation was skewed to the right. The transformed data were skewed to the left, but the skewness was lower than for the untransformed data, implying that it was closer to normality.

Table 3.1. Means, maximum values, and skewness for untransformed and transformed faecal egg count measured from 8 to 24 weeks of age. Phenotypic standard deviations are shown for the transformed data.

Age in weeks	8	12	16	20	24
Untransformed Data					
Mean	259	436	270	220	314
Max	3500	3200	2650	2900	4450
Skewness	3.86	1.71	1.91	2.88	2.99
Log-transformed Data					
Mean	5.22	5.67	5.00	4.85	5.11
Max.	10.5	8.08	7.89	7.98	8.41
Skewness	-0.53	-0.89	-0.21	-0.24	-0.13
σ_p†	0.91	0.94	1.10	1.10	1.03

† σ_p is the phenotypic standard deviation

The variance components estimated with the random regression model are shown in Figure 3.1. It can be seen that the additive genetic variance increases as the lambs grow older. The individual animal environmental variance does not show a simple trend over time. By interpolation it decreases in the beginning then increases, stabilizes for a relatively long period and then it decreases. It should be remembered that a first-degree polynomial was fitted for estimating the genetic component but a cubic polynomial was fitted for estimating the individual animal environmental component. For the residual variance only five values

were available, corresponding to the five actual measurements analysed. The rest of the points were interpolated using a weighted average, with respect to the neighbouring variance estimates. Thus the interpolated residual variance changed smoothly from one time point to the other. The litter effect was assumed to be the same for all sampling times, thus it is shown as a straight line.

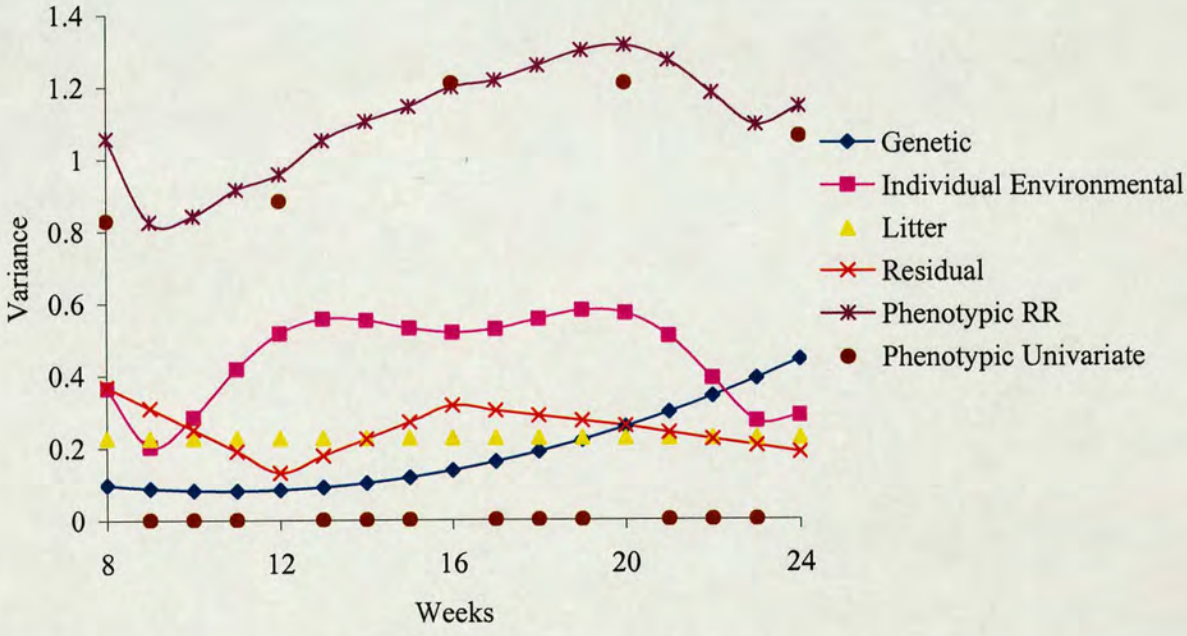

Figure 3.1 Variance components and the phenotypic variance for faecal egg count as estimated by a random regression model, and the phenotypic variance as estimated by univariate model.

In the same figure the phenotypic variance as estimated by the random regression model is shown along with the phenotypic variance as estimated by the within-time repeatability univariate model. There is reasonable agreement between the phenotypic variance estimates of the two models, although there is a tendency for the random regression model to estimate a slightly higher phenotypic variance.

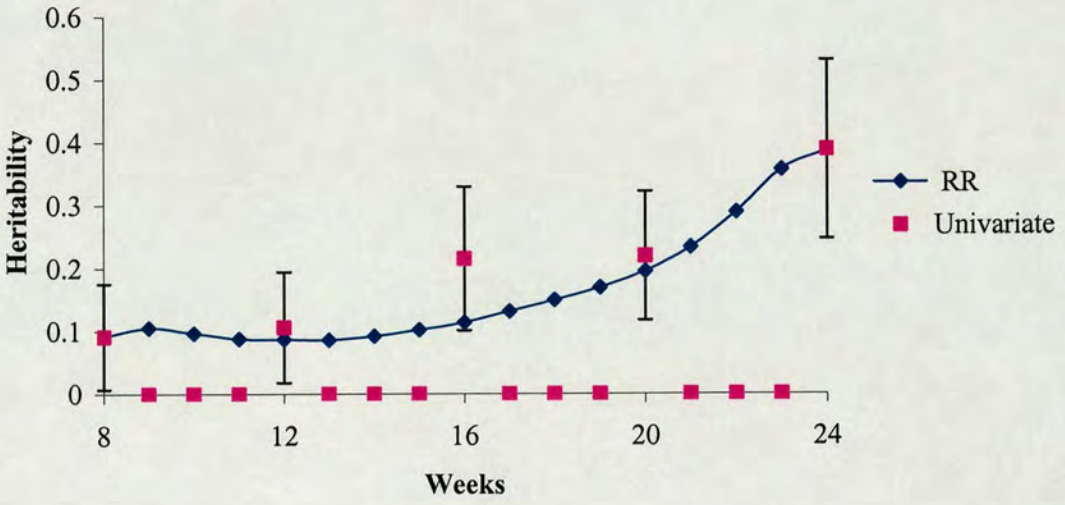

Figure 3.2 Estimates of heritability for faecal egg count from the random regression model and the univariate repeatability model. The bars represent s.e.

In Figure 3.2 the heritabilities estimated by the random regression model and the univariate repeatability model (along with the standard errors for the latter) have been plotted. As can be seen, the estimated heritabilities of the two models agree quite well, except for the sixteenth week for which there is a distinct discrepancy between them, although they are not statistically significantly different). For this specific time point the univariate model estimates a litter variance component of zero, which is not the case for the estimates of litter effect for the other sampling times. The heritability estimate is thus probably inflated at this time point in the univariate analysis, assuming that the true litter variance component is greater than zero.

Figure 3.3 shows the genetic correlations are shown between faecal egg counts at different sampling points in the form of a contour plot. The tabulated ages on the axes are the actual sampling times at which data were available.

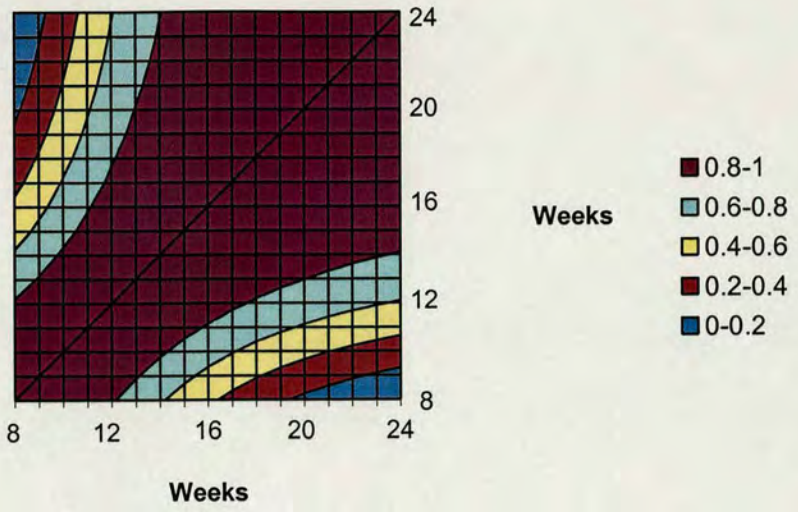

Figure 3.3. Contour plot of the estimated genetic correlation between faecal egg count measurements at different ages as estimated by the random regression model.

The estimated genetic correlations agree with *a priori* expectations, with measurements close in time having a higher genetic correlation which diminishes as sampling times become further apart. However, the decline of the genetic correlation becomes quite rapid once the sampling times are far apart and the genetic correlation of measurements taken in the first sampling time have an almost zero genetic correlation with samples of faecal egg count samples taken four months later. Visualising in three dimensions the above contour plot, it would be like a relatively wide plateau, which at its edges turns into a significant decline. As an approximate summary, sixty per cent of all the possible pairs of sampling times have a correlation greater than or equal to 0.8. We can arbitrarily assume that measurements that have a genetic correlation greater than 0.8 are genetically the same trait. According to this criterion, all measurements taken after fourteen weeks of age are genetically the same trait.

In Figure 3.4 the phenotypic correlation between faecal egg counts taken at different sampling times are shown. The phenotypic correlations between different measurements are lower than the genetic correlation and in no case do they exceed 0.65. Also, their pattern is

much more complex than the genetic correlations. This is especially the case for the early measurements, where small changes in time lead to relatively large changes in the value of the correlation. In large sectors of Figure 3.4 the correlations are very low (<0.3) and sometimes they are slightly negative.

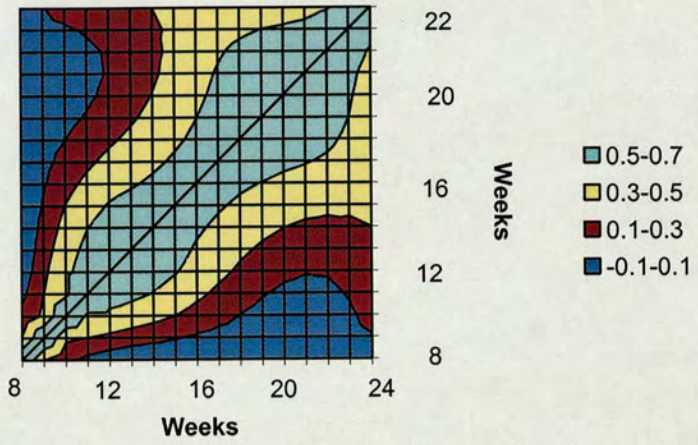

Figure 3.4. Contour plot of the estimated phenotypic correlation between faecal egg count measurements at different ages as estimated by the random regression model.

3.4 Discussion

The aim of this chapter was to obtain a good description of the age-dependent genetic properties of faecal egg count data from a population of lambs. Changes in the genetic and phenotypic parameters in this dataset were studied over time and as a result of the properties of the random regressions, an estimate of the best sampling time within this period was obtained.

The heritability estimates obtained by the random regression model and the univariate within-time repeatability model are similar at all ages with the exception of the estimate at sixteen weeks of age. This discrepancy is most probably due to the different way these models treat the litter variance. In the random regression model, it is assumed to be constant over the time period examined. Strictly speaking the litter effect should be allowed to change. This was attempted but the analysis failed to converge. In the univariate repeatability model the litter effect variance component estimate was zero, which is biologically unlikely, especially as this point coincides with weaning and the estimate of the same component at later time points was higher. The implication of a zero estimate for the litter variance component is that true litter variation was partitioned towards the genetic component, resulting in an overestimate of the heritability. Whilst a constant litter effect is not the most satisfying assumption, the resulting genetic variance and heritability estimates are consistent with reasonable *a priori* expectations, giving confidence in the plausibility of the random regression approach for this dataset.

Bishop *et al.* (1996) analyzed a subset of this dataset. Their estimates of correlations differ from the estimates in this study, without any apparent pattern in the way in which the correlations differ. These differences could be attributed to either a) the fact that the current dataset is expanded by one more year of data or b) the different transformation [ln(Fec+25)]

used compared with Bishop *et al.* (1996) [ln(Fec+1)]. Given the large standard errors of the estimated correlations of the above authors a difference in the estimates is not surprising. However, the different transformations do have an effect on the results and this effect can be seen in Table 3.2 where univariate heritability estimates obtained from the current dataset are shown for ln(Fec+1) and ln(Fec+25). The transformation used in the current analysis (ln(Fec+25)) results in a distribution closer to the normal distribution and also a more consistent pattern of change in the estimated heritabilities with age.

Table 3.2 Heritability estimates using two different transformations, results obtained from univariate analyses.

	Heritability	
Age in weeks	ln(Fec+1)	ln(Fec+25)
8	0.14	0.09
12	0.12	0.11
16	0.19	0.22
20	0.16	0.22
24	0.28	0.39
average s.e.	0.10	0.11

The contour plot of the genetic correlations between time points in Figure 3.3 can be split into three arbitrary periods. The first is the period of the life of the animal up to week twelve for which the samples taken at different sampling times have a genetic correlation greater than or equal to 0.8 with faecal egg count measurements taken at week eight of the animals' life. A second period which starts at week fourteen, may be defined as that which starts as soon as the faecal egg count taken at the specific sampling time has a correlation greater than or equal to 0.8 with the faecal egg count sample taken when the animal is 24 weeks old (last sampling time). This leaves a third small transition stage, i.e. from 12 to 14 weeks. This pattern agrees with the acquired nature of resistance to gastrointestinal parasites (Stear *et al.*

1999) where immunity is age dependent and is mounted gradually after the animal is challenged. Furthermore, it can be deduced that measurements of faecal egg count taken after the third month of life of the animal (i.e. at week 14), can be treated as the same trait. However, measurements taken at week 14 have a relatively low heritability and as it can be seen in Figure 3.1 there is a tendency for the genetic variance to increase with time. The heritability reaches its maximum value (within this time period) at six months of age. Therefore the best sampling time for faecal egg count would be when the animal is six months old. Earlier measurements would be measuring essentially the same trait, but would be less effective in terms of genetic progress, as the heritability is lower.

Random regression models for estimating genetic and phenotypic parameters have been applied mainly to growth (Meyer 1999, Albuquerque and Meyer 2001) and milk yield (Jamrozik *et al.* 1997, Kettunen *et al.* 2000, Jensen 2001) in cattle. The pattern of genetic correlations predicted by random regressions for these cattle traits differs markedly from the pattern of genetic correlations for faecal egg count in our analyses. The genetic correlation for these traits stays, in general, high (>0.80) for a longer time period than they do for faecal egg count. The decline in the genetic correlation is also more rapid for faecal egg count than the above production traits. The above pattern is also observed for the phenotypic correlation. Our results may be interpreted as showing the development of immunity across time (as assessed by the genetic correlation and heritability pattern) and also the complex patterns across time (as assessed by the phenotypic correlation patterns).

In conclusion, the random regression model has provided an adequate and informative description of the data. It has the advantage, compared to other models, that it allows genetic and phenotypic interpolation from the data allowing us to obtain a better understanding of the behavior of the genetic and phenotypic parameters in the time period for which data are

available. These in turn allow assessments to be made of the impact of measuring lambs at different ages on overall genetic progress.

4. *In silico* quantification of the combined effect of long term selection with separation or culling of the lambs with the highest faecal egg count, in grazing lambs.

4.1 Introduction

As described in Chapter 1, sheep grazing in extensive production systems are almost certain to be infected by gastrointestinal nematode parasites. In temperate regions they usually pose no life threatening disease for the infected animals and are treated with anthelminthic drugs. However, they cause great production losses (measured as live-weight) which Coop *et al.* (1985) estimated to be in excess of 25% in UK conditions.

Several methods have been proposed for overcoming the problem of parasitism with lower usage of anthelminthics (or maybe in the future no anthelminthics at all). These approaches include management, biological control, vaccine development, nutritional supplementation and selection of sheep for resistance to nematode parasites. All these approach the same problem from a different angle and have their benefits and their drawbacks. In this chapter we will concentrate on selection of animals for resistance to nematodes aided by the culling of heavily infected animals.

The trait usually used to select animals for resistance to nematode infections is faecal egg count. This trait is interesting as it is both an indicator of relative levels of resistance, as well as a predictor of the future pasture contamination. Therefore, it has been predicted that selection for increased resistance to gastrointestinal parasites will affect the epidemiology of the disease (Bishop and Stear 1997,1999). In their simulation study, these authors estimated that the response to selection as predicted from quantitative genetics theory could be an underestimate, with observed responses being as much as double those predicted. This is due to the fact that quantitative genetics theory, when naively applied, does not account for the change in the infection transmission dynamics i.e. the epidemiology of the disease. This

hypothesis has now been verified experimentally by Leathwick *et al.* (2002). It should be stressed that this extra response is due only to environmental factors. However, in a commercial farm situation, the primary aim is an increase in production irrespective of whether this increase is due to genetic or environmental factors. The environmental benefits of selection for resistance to worms are important and one should try and exploit them, along with the genetic benefits, as much as possible. This is different from more 'conventional' traits like milk yield and backfat where the genetics and the environment are generally independent. On the contrary, with heritable disease resistance the genetics of the host (and the parasite) affect the environment in which the animals live, as field (Bisset *et al.* 1997) and simulation (Bishop and Stear 1997, 1999) studies have shown.

A distinct feature of faecal egg counts is that they are not normally distributed. Faecal egg counts are heavily skewed to the right and often follow a negative binomial distribution. In this case, a few individuals harbour the majority of the parasites: as a rule of thumb, 80% of the eggs are attributed to 20% of the animals (Bishop and Gettinby, 2000). It should be noted that in the studies described in this thesis the data did not, in general, fit the negative binomial distribution. Even when faecal egg counts do not fit the negative binomial distribution they are nevertheless highly skewed and a small proportion of the animals contribute most of the pasture contamination. In a trait with so highly skewed a distribution, culling or separation of the "wormiest" animals may have a dramatic effect on the contamination of the pasture, assuming that the trait is repeatable. Whereas selection for resistance to nematodes may have a between-year effect, i.e. beneficial effects will cumulate over time, culling may well have an environmental effect within-year and, depending on the management, cumulative effects across years as well.

Furthermore, given the problems of assigning an economic value to resistance and the distribution of faecal egg count, culling or separating the "wormiest" animals might be a first step in incorporating resistance to nematodes into a breeding program. A two-stage selection could be implemented where animals are culled/separated as soon as possible on faecal egg counts and then selection is practiced on the remaining animals for productivity or resistance. The difference between this approach for faecal egg counts and that of two-stage selection for other traits is that an immediate environmental effect is to be gained from the separation/culling procedure.

In this chapter the effect of separating/culling the "wormiest" animals of a flock on their egg counts will be considered using computer modeling (Fortran 90). As a basis the model initially developed by Bishop and Stear (1997, 1999) will be used. Different scenarios of separation/culling levels under various levels of repeatabilities (of faecal egg counts) will be explored. The effect on productivity and on the epidemiology of the disease will be explored and quantified both for short and long-term selection under different management regimes.

4.2 Materials and methods

4.2.1 General framework

The principal aim of this chapter is to examine, by means of simulation, the effect of separating/culling the "wormiest" animals, both within and between-years. Culling and separation are defined below. As discussed in Section 4.1, resistance to worms as expressed in the form of faecal egg counts is moderately heritable and has a skewed distribution. Moreover, current faecal egg counts determine future parasite challenges. Thus, the environmental effects of selection are important, in contrast with most other traits.

Separating a small percent of the "wormiest" animals should affect the epidemiology of the disease within a grazing season. The "wormiest" animals contribute a large proportion of the parasite eggs to the pasture so when these animals are separated from the flock this major contamination source is removed. Therefore there are fewer parasite eggs going onto the pasture and fewer larvae will be available for ingestion by the rest of the flock which consequently may become healthier and may have lower production losses. In this justification of separation there is an important underlying assumption: the trait for which the separation/culling is practised has to be repeatable. That means that the animals which have the most parasites in a given sampling time, will have high numbers in subsequent samplings. If the trait is not repeatable then animals which where ranked as the worst at one sampling time will not on average be those ranked as the worst at subsequent sampling. It is not a prerequisite for separation/culling to work that the trait has to be heritable. In this chapter however, and in accordance with field studies, the heritability of faecal egg counts will be assumed to be closely related to the repeatability of the trait.

Separation and culling may be defined as two different management practises. Both involve dividing the flock into two parts: the 'healthy' and the 'wormy'. 'Healthy' in this context has

not the meaning of disease free but merely the part of the flock with the lower degree of parasitism. In both practices a faecal egg sample is taken at a given time point and the decisions on separation or culling are taken as soon as the results of the faecal egg counts are available from the laboratory. In the separation practice the wormiest part of the flock is separated in the same pasture by fence from the healthy part of the flock, for example by using mobile fences. In this chapter, the separation of the animals is proportional to their number, i.e. if 15% of the wormiest animals are to be separated they will be given 15% of the pasture and the healthy part of the flock 85% of the pasture. Therefore the stocking density of animals remains the same as before the separation took place. In the case of culling, as soon as the results of faecal egg counts are available, the wormiest part of the flock is culled. The remaining animals can use and feed on the whole pasture and the stocking density of the animals decreases. This has implications for the disease epidemiology as the probability of larvae ingestion decreases. Consequently the animals are expected to have lower worm burdens and be healthier.

4.2.2 Model

The model used was developed from that of Bishop and Stear (1999). In this model the level of challenge for each animal is related to its food intake. The food intake is correlated with the live-weight and increases with age. The host-parasite interactions are defined through three distinct and, for simplicity, uncorrelated traits, which are assumed to be under the genetic control of the host. These traits are establishment of the parasites, development of the parasites and fecundity of the parasites. Weak density dependent effects were assumed, i.e. declining per capita parasite fecundity with increasing parasite numbers. The free-living stages of the parasites were modeled in terms of mortality rates and probability of ingestion. The combination of the three host traits (establishment, fecundity and mortality) along with a measurement error result in the observed faecal egg counts, as shown in Figure 4.1.

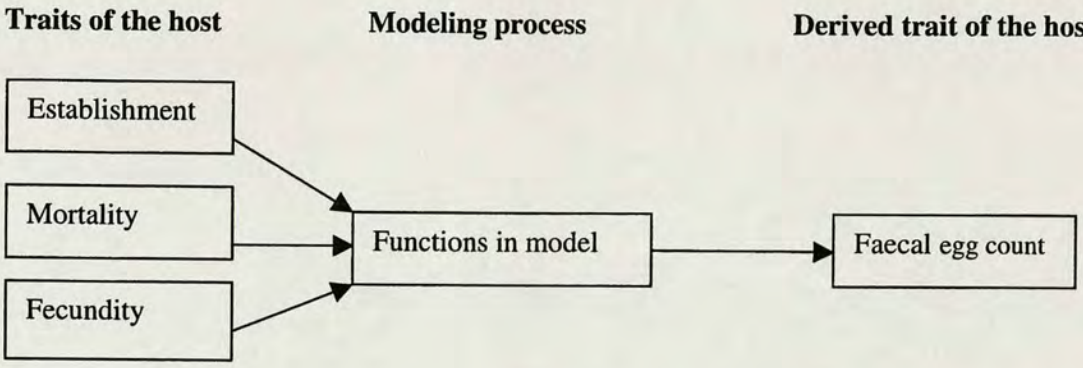

Figure 4.1 Pictorial representation of the derivation of faecal egg counts.

The infection of the animals by parasites is expensive, in production terms, to the animals. These production losses result from decreased host appetite and the decreased efficiency of utilization of the energy available for metabolism (van Houtert and Sykes, 1996; Coop and Holmes, 1996). Furthermore, there is evidence for production losses in lambs associated with larvae intake *per se* (McAnulty *et al.* 1982) which might be the result of the cost and the consequences of the immune response. Therefore, the production losses can be modeled as a function of cumulative larvae intake and cumulative worm burden with coefficients derived using a procedure outlined by Leathwick *et al.* (1992). In this model the production losses have been modeled as a function of individual larval challenge and worm burden, calculated as a function of the product of worm number and worm size. The coefficients used were the same as in Bishop and Stear (1999). The potential live-weight can then be adjusted for the production losses caused by parasites to predict the actual observed live-weight.

4.2.3 Model parameters

The input parameters of the model were chosen to be similar to those estimated from experimental data, and the outputs for achieved live-weight and faecal egg counts mimicked field data. Lambs were assumed to have an average potential live-weight of approximately

40 kg at six months of age in the absence of parasitism. Production losses in this model were estimated as a function of cumulative larval intake and cumulative worm burden in accordance with Leathwick *et al.* (1992). Furthermore, Stear *et al.* (2002) suggest that the actual worm size may be important in the pathogenesis of infection in addition to worm number *per se*. Therefore, the worm mass was calculated as the product of worm number and worm size. The production loss penalty was scaled so that the productivity losses under conditions of no anthelminthic treatment were 25% of the live-weight gain. Anthelminthic treatment restores some, but not all of the production losses and the estimate of Coop *et al.* (1985) was that it restores less than 0.30 of the productivity losses caused by parasitism. Using this as a conservative estimate, anthelminthic treatment in this model was modeled to restore proportionately 0.33 of the productivity loss at six months of age. Lambs were treated with anthelminthic at three, four and five months of age. Following anthelminthic treatment average production losses were 0.167 % of live-weight. The average average live-weight achieved following anthelminthic treatment and the imposition of live-weight gain losses was 33 kg.

The mean values and coefficient of variation for fecundity, establishment and mortality, were the same as those used by Bishop and Stear (1997, 1999). Weak density dependent effects, as described by Bishop and Stear (1997), were assumed (b=-0.25). The density coefficient b, can take values from 0 representing total absence of density dependent effects to –1 representing complete density dependent effects, such that egg output is independent of worm burden. The heritabilities of mortality and establishment of the parasites were both 0.2, as in Bishop and Stear (1999). Live-weight and food intake, under parasite free conditions, were assumed to have heritabilities of 0.2, permanent environmental effects of 0.15, coefficients of variation of 0.1 and 0.2, respectively and correlations (both genetic and phenotypic) of 0.5, as used by Bishop and Stear (1999). For fecundity, and for reasons

explained in a later section connected with the overall heritability of Fec, the heritability in some cases varied from the value of 0.5 used by Bishop and Stear (1999) to 0.8.

4.2.4 Repeatability

Different repeatabilities between three and six months of age were modeled for faecal egg counts in this study. Because faecal egg count is a highly skewed trait, a suitable transformation had to be applied to convert the data to normality. The logarithmic transformation was used for all the parameter estimations since it is the most common transformation encountered in the literature and converts the trait to an approximately normally distribution. Faecal egg counts were assumed to become heritable after three months of age. The repeatability sets (under some assumptions: equal variances, and r_g=1, see Falconer and Mackay, 1997) an upper limit for heritability. The parameter space of repeatabilities explored was 0.2 – 0.6 in steps of 0.1. The repeatability of faecal egg counts was manipulated by two methods: firstly by varying the faecal egg count measurement error, a component of the environmental variance of the trait. However, when the measurement error was set to zero the repeatability of the trait was approximately 0.45. Higher repeatability values were achieved by an increase in the heritability of fecundity, because the value used by Bishop and Stear (1997, 1999) is conservative (Stear *et al.*, 1997b). Therefore, for repeatabilities of Fec of approximately 0.5 and 0.6, the heritability of fecundity was increased to 0.65 and 0.80 respectively. As an example, when the repeatability was approximately 0.2 the heritability of this trait was 0.18. In this case the genetic correlation between measurements taken at three and six months of age was 0.92 and the phenotypic correlation was 0.22. The equivalent parameters for repeatability of 0.6 were 0.50, 0.95 and 0.61 respectively. It should be noted that these parameters are estimated from the model outputs, and they are the means of such estimates for ten replicates. As for all cases for

parameter estimation (described below) a flock of 5000 ewes was simulated producing 10000 lambs.

4.2.5 Separation and Culling

The purpose of this study was to assess the effect of separation/culling of different proportions of animals, using faecal egg counts as the indicator trait for resistance to gastrointestinal parasites, in combination with selection either for increased live-weight or reduced faecal egg counts. The separation/culling took place ten days after the faecal egg sample was taken (90 days of age) allowing time for the sample to reach the laboratory, be processed and results returned to the farm. Two scenarios were explored. Firstly the animals with the higher faecal egg counts were assumed to be confined in a separated part of the pasture proportional to their number and therefore the density of the animals remained the same. The wormiest animals were thus independent from the rest of the flock and did not contribute to the contamination of the pasture. Secondly, in the case of culling, the animals with high faecal egg counts were culled ten days after the samples were taken and the rest of the flock continued to graze the same pasture. Thus, there was different recontamination of the pasture due to nematode eggs and therefore different subsequent levels of larvae intake in the two scenarios. The separation/culling levels varied from no separation/culling to 5%, 10% and 15% of the animals with the higher faecal egg counts separated or culled. The no separation/culling scenario was the control.

Estimation of the genetic parameters, using the ASREML package (Gilmour *et al.* 1996), was performed to explore if culling had an effect on the genetic parameters and the correlation between the three 'major' traits: live-weight (Lw), food intake (FI) and faecal egg count (Fec) transformed to the logarithmic scale. In each case the simulated flock comprised 5000 ewes mated to 250 sires and each ewe produced twins. Twenty replicates were run for

each scenario. In the case of the control the estimation of the genetic parameters was performed using the full dataset of the lambs. In the separation cases the target proportion of the "wormiest" animals were excluded from the analysis since the main interest was in the part of the flock which would contribute to the next generation.

4.2.6 Selection and management.

The purpose of this study was to assess the combined effect of selection and culling (or separation) for faecal egg count. Selection was applied to males at six months of age and they were used for mating at seven months of age. No direct selection was applied to females. The selection criterion was either the geometric mean faecal egg count from measurements taken at four, five and six months of age (referred to as the mean faecal egg count unless otherwise stated) or the achieved live-weight at six months of age. The simulated flock was in this case a flock of five hundred ewes randomly mated to twenty-five sires. Each ewe was assumed to give birth to twins. Therefore, the top 25 sires out of 500 were selected by phenotypic truncation selection.

Two grazing management situations were explored. In the first, the lambs faced the same level of challenge each year. In the second situation, the level of infection faced by the lambs in the beginning of each year was directly proportional to the faecal egg count mean of the previous years. This is the situation where carry-over effects are imposed as described by Bishop and Stear (1997). Therefore, four different situations were explored for each separation/culling level: selection for increased live-weight with no carry-over effects, selection for live-weight with carryover effects, selection for reduced faecal egg counts with carry-over effects and selection for faecal egg counts with no carry-over effects. For each combination of selection-management and separation/culling level ten replicates were run. In every case the level of pasture larval contamination was monitored.

4.2.7 Interpretation of the results

The response to selection was decomposed to its components i.e. the genetic and environmental components. This was done using the means of the first and the second year of selection on the logarithmic scale for faecal egg counts. The logarithmic scale was used because the genetic parameters available were on this scale. This was done for repeatability of faecal egg counts of 0.6, for all separation levels and for the management regimes with no carryover effect management regimes. The decomposition of the response was also performed in the same cases as the above mentioned for live-weight, which is the production trait of interest. Due to the fact that separation of animals was practised, the environmental response can be further decomposed to two components: the effect due to selection and the effect due to separation. These components are inseparable in the case of a real flock, but in a simulation study, the management can be altered in such a way that one always has the results for the same replicate but with various management techniques. It was therefore possible to estimate these different environmental components. The methodology is summarized in Table 4.1.

Table 4.1 Method for estimating environmental components of response to selection, '0' indicates no separation or culling, '1' indicates that separation has been applied

	Base Population	1^{ST} Generation
Mean 1	0	-
Mean 2	1	-
Mean 3	1	1
Mean 4	1	0

Mean 1 is the mean of the base control population. Mean 2 is the mean obtained when separation is practiced for the base population i.e. the 5%, 10% or 15% of the wormiest animals are separated from the rest of the flock. The difference between these two means is the effect of separation in the base population. Mean 3 is the mean obtained when separation

is practiced in the first generation and in the base population. Mean 4 is the mean obtained when separation is practiced in the base population but not in the first generation. The difference between mean 4 and mean 3 is the effect of culling at the first generation on the same generation. The expected genetic response is estimated from quantitative genetics theory using the genetic parameters estimated from the control base population. The environmental response is then estimated as the difference between the observed response without separation of the wormiest animals and the estimated genetic response.

4.3 Results

The results will be presented in detail for repeatabilities of faecal egg counts of 0.2 and 0.6. The study was also performed for repeatabilities of faecal egg counts of approximately 0.3, 0.4 and 0.5, and all the results lay more or less linearly between those for repeatabilities of 0.2 and 0.6. Initially the results for all separation scenarios will be presented and considered.

4.3.1 Within-year effect – generation zero

The within-year effect of separation on the egg output was quantified. Because this was a simulation study the true individual parasite egg output for every animal was available. This quantity is different from the estimated faecal egg counts due to their measurement error and to the fact that faecal egg counts are adjusted for faecal output. The true egg output is what really happens, whereas faecal egg count is an indication. The mean of ten replicates from the remaining [1000*(1 – proportion separated)] animals are given in Figure 4.2 for true eggs for different separation levels and repeatability of faecal egg counts of 0.6 and for repeatability of 0.2 in Figure 4.3. There are two significant drops of egg number in the faeces to zero, at days 120 and 150 due to drenching at these days. It is assumed that the anthelminthic kills only the mature worms and only acts for one day, so mature worms will appear from the next day after drenching. It can be seen that in both cases the separation of animals leads to slightly decreased recontamination of the pasture for both levels of repeatability. As expected, the differences between the different separation levels were greater when the repeatability was 0.6 than when it was 0.2. In the case of repeatability of 0.6, at the 5% separation level the average difference between true faecal egg output and the control was 4.5% at six months of age increasing to 8.5% and 12.5% difference in the 10% and 15% separation levels respectively. In the scenario with repeatability of 0.2 the 5% separation level had a 2.5% difference from the control while the 10% and 15% had a 4.5% and a 6.5% difference, respectively. It should be noted that in the first year the distribution of

faecal egg counts was not as skewed as the faecal egg counts distributions observed in field studies. This was due to the fact that in the base population the combination of the three distributions of mortality, fecundity and establishment, did not result in a distribution as those seen in field conditions. The coefficient of skewness was 1.33 compared with a typical value of 3.50 from field data (Chapter 3).

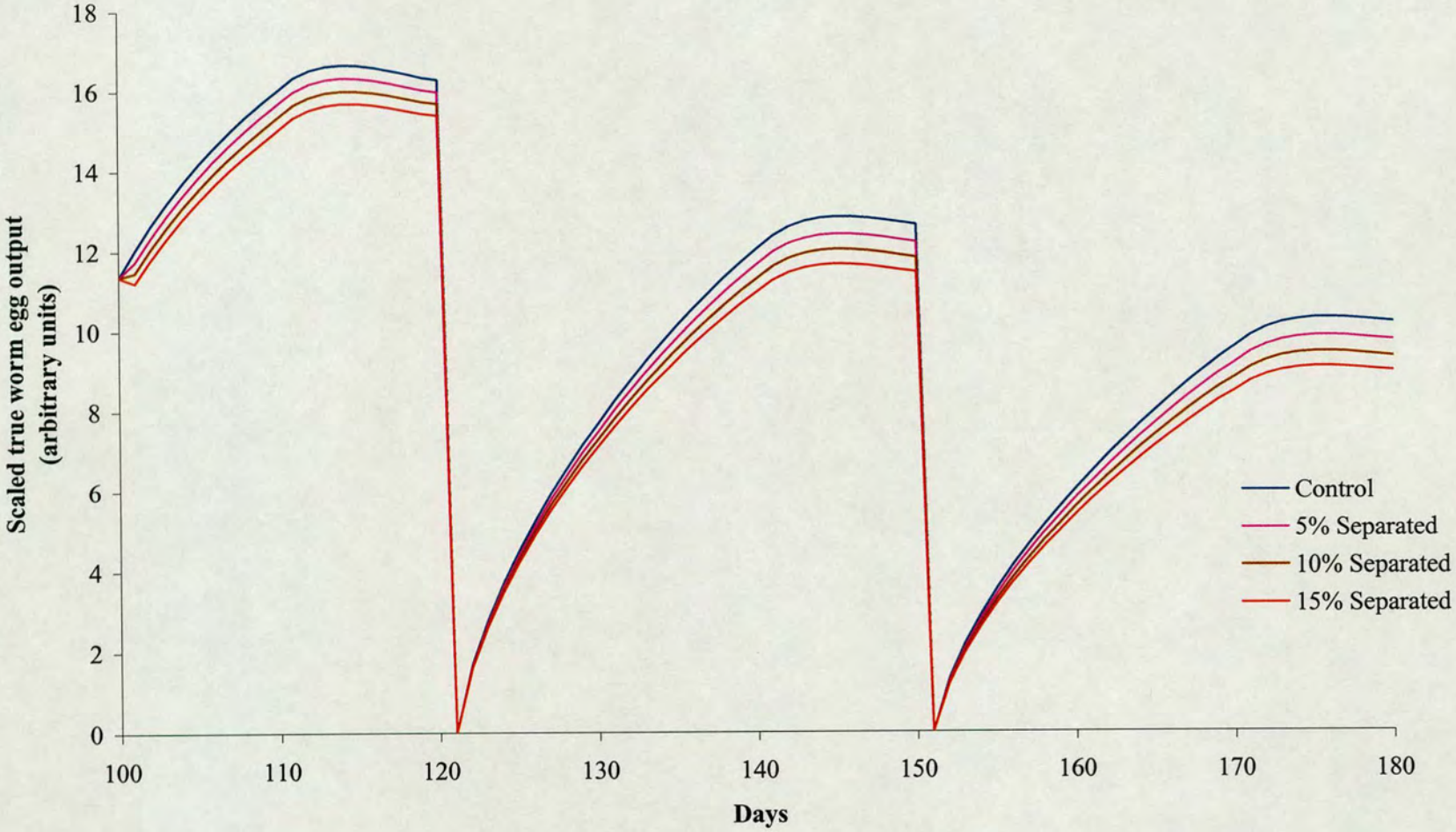

Figure 4.2 True egg output for the animals remaining after separation. Repeatability of faecal egg counts is 0.6

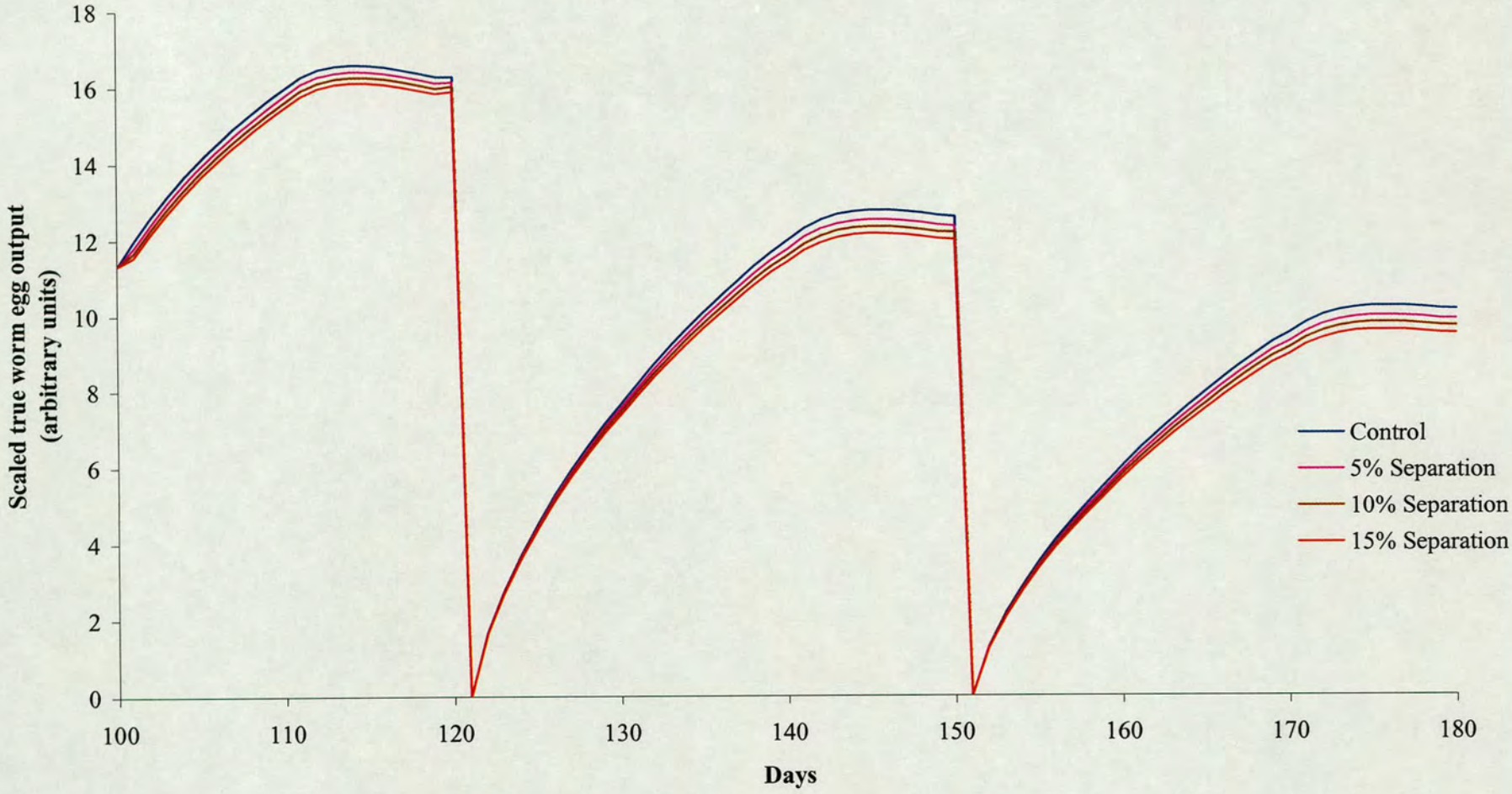

Figure 4.3 True egg output for the animals remaining after separation. Repeatability of faecal egg counts is 0.2

In Table 4.2 the effects of different levels of separation on faecal egg count and live-weight in the zero generation, both observed from simulation and theoretically estimated using both trait distributions, are presented for a faecal egg count repeatability of 0.2. The results show the faecal egg count and live-weights of the remaining animals, i.e. not separated out, expressed as a difference from the control. The percent difference in the faecal egg count between the separation and control scenarios is relatively high whereas the difference for live-weight is trivial. For both cases the observed differences are higher than those theoretically estimated. This is due to the fact that simple quantitative genetics estimates do not account for the epidemiology of the disease, i.e. the changing pasture contamination during the season.

Table 4.2 Effect of different levels of separation based on Fec, both on Fec and Lw, in the zero generation. Repeatability of Fec = 0.2

	Effect on Fec (%)		Effect on Lw (%)	
% separated	Observed*	Estimated	Observed	Estimated
5%	-2.2	-1.4	0.19	0.05
10%	-3.7	-2.1	0.39	0.13
15%	-4.9	-3.5	0.55	0.22

*observed from simulations

The equivalent results are presented in Table 4.3 for repeatability of faecal egg counts of 0.6. As can be seen, the results are qualitatively similar to those of Table 4.2 although the magnitude of the differences is bigger.

Table 4.3 Effect of different levels of separation based on Fec, both on Fec and Lw, in the zero generation. Repeatability of Fec = 0.6

	Effect on Fec (%)		Effect on Lw (%)	
% separated	Observed	Estimated	Observed (%)	Estimated
5%	-3.3	-2.5	0.41	0.21
10%	-6.0	-5.2	0.72	0.42
15%	-8.6	-6.9	1.01	0.59

4.3.2 Long term response, when combining separation with selection for either faecal egg count or live-weight.

4.3.2.1 Selection for faecal egg counts. No carryover effects

In Figure 4.4 the long-term results are given for combining selection for low faecal egg count and separation, with an assumed repeatability of faecal egg counts of 0.2. All the three different separation levels are shown along with the control line. In this particular case the control line generally has the lowest faecal egg counts; of the other lines the 15% separation level was closest to the control followed by the 10% separation level and the 5% separation level.

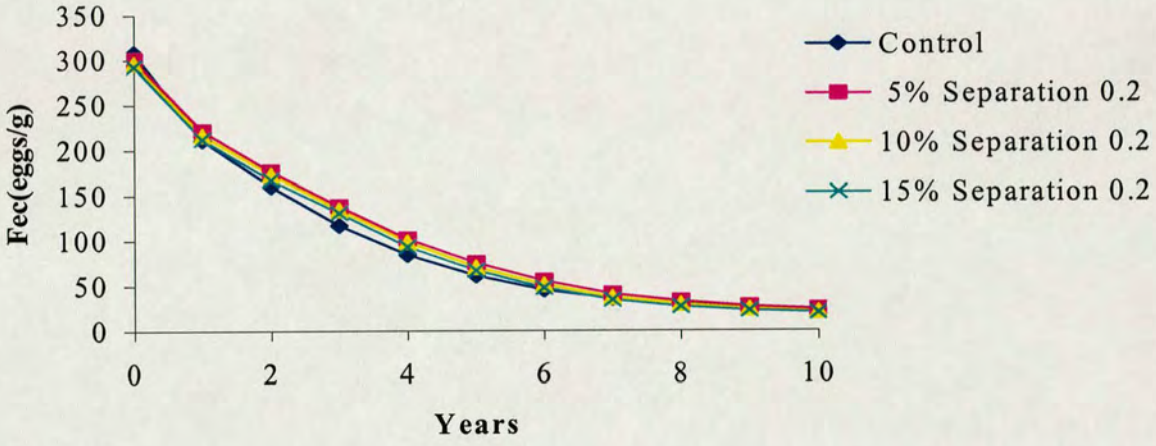

Figure 4.4 Long term response for all separation levels when selecting for reduced faecal egg counts. Repeatability of faecal egg counts 0.2

The three separation levels had 2.2%, 3.7% and 4.9% lower faecal egg counts than the control at the tenth generation. It should be noted that the different separation levels had lower faecal egg counts in the zero generation but from then onwards the faecal egg counts in the separation became higher than the control faecal egg counts. However after some generations this difference diminished as can be seen in figure 4.4. When the repeatability of

faecal egg counts is 0.2 it is apparent that: i) the culling effect is quite small, ii) separation removes animals (or places them in a more contaminated part of the pasture) some of whom might otherwise have been selected. These two factors combine to give a small effect in generation zero, and a counterintuitive effect subsequently. If the repeatability is higher, then: i) the culling effect is expected to be greater and ii) there is less risk of separating animals, some of which might otherwise have been selected.

Figure 4.5 shows results of separation and selection at a repeatability of 0.6, with previous results for repeatability of 0.2 included for comparison.

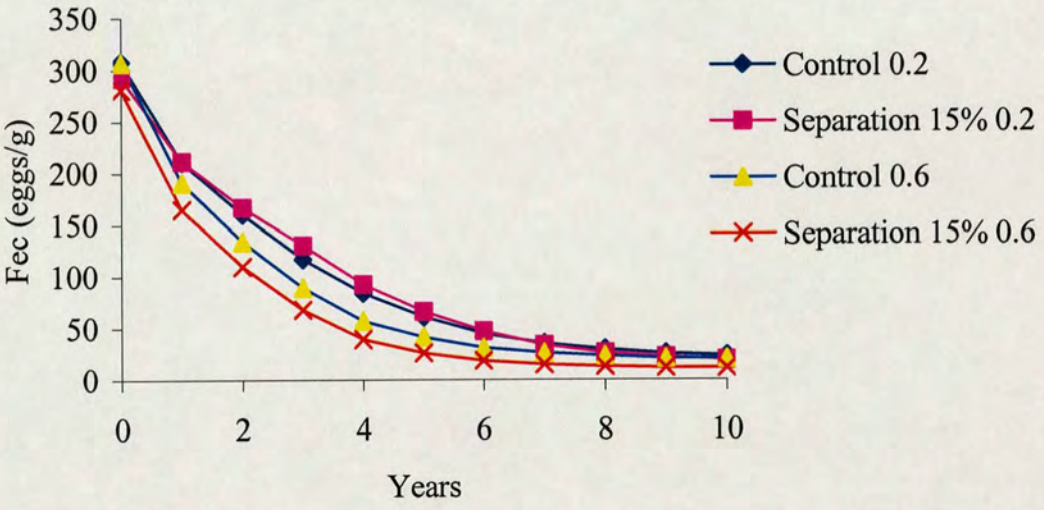

Figure 4.5 Long term response in faecal egg counts when selecting for faecal egg counts. Repeatabilities of faecal egg counts are 0.2 and 0.6. Control and 15% separation level shown for both cases

For repeatability of 0.2 the estimates from the simulation, at the tenth generation, of faecal egg count expressed as a deviation from the control were -3.3%, -6.0% and -8.6% for -5%, 10% and 15% separation levels, respectively. When the repeatability of faecal egg counts

was 0.6 the faecal egg counts of the 85 to 95% remaining animals at the different separation scenarios were always lower than the control. The range was between 3.3% lower for the zero generation of the 5% separation scenario to 45% lower for the tenth generation of the 15% separation case. However, when the repeatability of faecal egg counts was 0.6, the 5%-15% worst animals in the separation scenarios had higher faecal egg counts than the control scenario, ranging from 46% for the zero generation of the 15% case to 321% in the tenth generation of the 5% case. Overall, in the tenth generation, the animals in the 5% separation case had 4.4% lower faecal egg counts compared with the control, in the 10% separation case 8.0% lower faecal egg counts and in the 15% separation case 17.1% lower faecal egg counts.

The correlated response for live-weight is given in Figure 4.6 for the above-described cases. For repeatability of faecal egg counts of 0.2, the difference between the control and the separation was less than 1% in all cases and in variable directions.

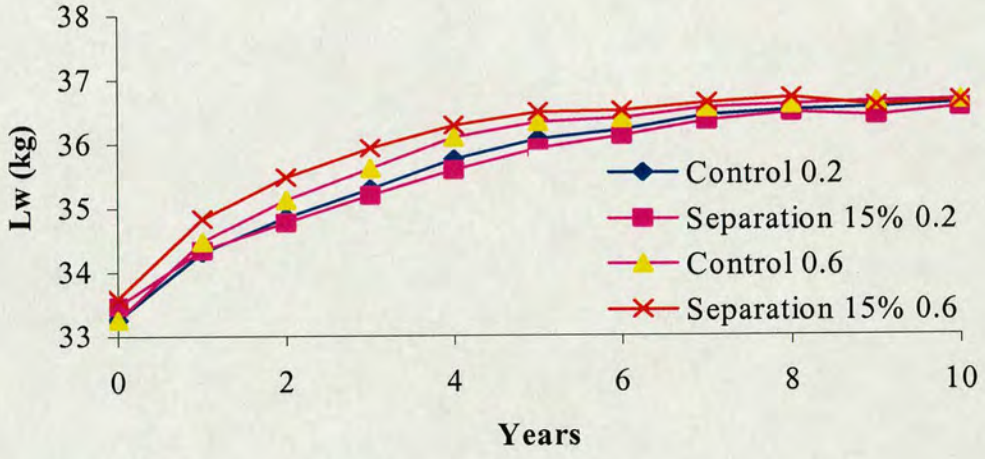

Figure 4.6 Long term correlated response in live-weight when selecting for reduced faecal egg counts. Repeatabilities of faecal egg counts: 0.2 and 0.6. Control and 15% separation level shown for both cases

When the repeatability of faecal egg counts was 0.6 all separation levels had slightly higher live weight compared with the control. In the zero generation the 5%, 10% and 15% separation cases had 0.4%, 0.7% and 1.0% higher live-weight than the control case respectively. At the tenth generation the respective differences were 0.5%, 1.8% and 1.8% respectively. When we look at the worst animals who were separated at the zero generation had –7.6%, -6.0% and –5.1% lower live weight compared to the control. This difference became smaller over years and at the tenth generation it was –2.9%, -3.0% and –0.01% respectively for the 5%, 10% and 15% separation cases respectively. On average, combining the two sub-populations means, for the zero generation the live weight was only marginally different from the control, for all separation cases. In the tenth generation the 5%, 10% and 15% separation cases had less than 1% difference from the control.

4.3.2.2 Selection for faecal egg counts. Carryover effects

In Figure 4.7 the faecal egg counts are shown for the control and the 15% separation level for of faecal egg count repeatabilities of 0.2 and of 0.6. As can be seen this closely resembles

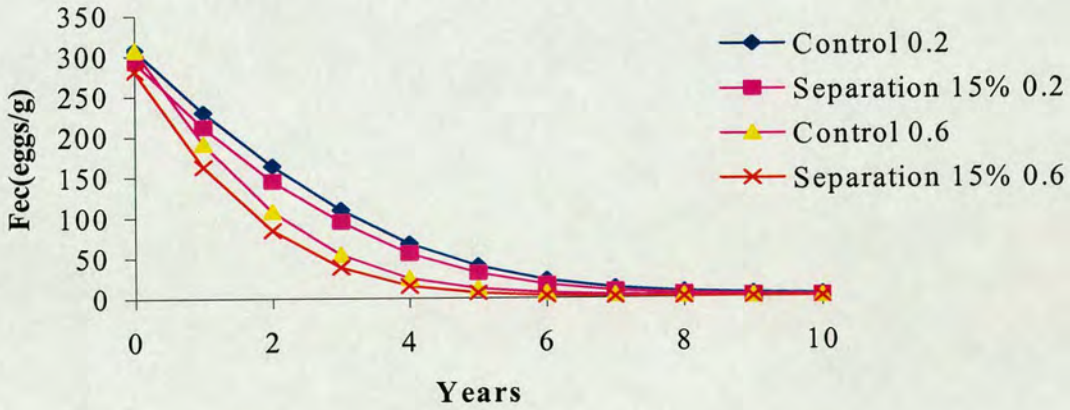

Figure 4.7 Long term response in faecal egg counts when selecting for reduced faecal egg counts, imposing Carryover effects. Repeatabilities of faecal egg counts: 0.2 and 0.6. Control and 15% separation level shown for both cases

the case of selection for faecal egg counts without carry-over effects imposed. The mean faecal egg counts when carry-over effects are imposed are lower than for the case when carry over effects are are not imposed, as expected and in agreement with Bishop and Stear (1999).

The correlated responses for live-weight for this case are given in Figure 4.8. As can be seen, it follows the same pattern as when carry-over effects are not imposed with the difference that the animals reach higher weights when these effects are imposed.

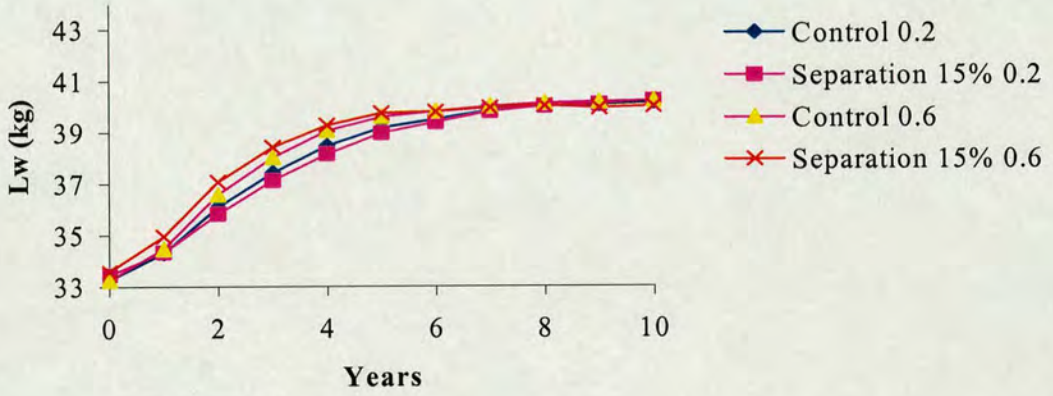

Figure 4.8 Long term correlated response for live-weight when selecting for reduced faecal egg counts. Carry over effects. Repeatabilities of faecal egg counts: 0.2 and 0.6. Control and 15% separation level shown for both cases

When the repeatability of faecal egg counts was 0.6, the worst animals which were separated always have lower live-weights than the control. The worst animals have 7.6% lower live-weight than the control in the zero generation in the 5% separation scenario, 6.0% lower live-weight in the 10% separation scenario and 5.1% in the 15% separation scenario. This difference diminished as the selection progressed and it became constant at the level of approximately –1% in the third generation for the 15% separation scenario and at the fifth generation for the 10% and 5% separation cases, respectively. The overall result combining

the results from the best and the worst separated animals was that the separation had a very small effect on the overall performance of the animals in the range of ±1%.

4.3.2.3 Selection for Live-weight. No carry-over effects

In Figure 4.9 faecal egg counts are shown for the case when selecting for improved live-weight, for the control and the 15% separation level for repeatabilities of 0.2 and 0.6.

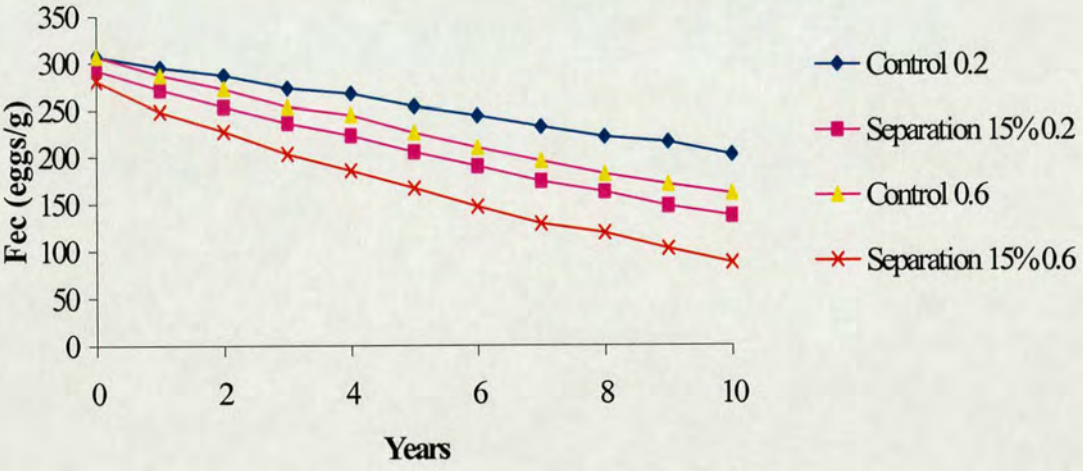

Figure 4.9 Long term correlated response in faecal egg counts when selecting for live-weight. No carryover effects. Repeatabilities of faecal egg counts: 0.2 and 0.6. Control and 15% separation level shown for both cases

In both repeatability scenarios (0.2 and 0.6), faecal egg counts in all three-separation cases were lower compared to the control, in all generations. When the repeatability of faecal egg counts was 0.2, for the non-separated animals the difference between the control and the separation scenarios increased over generations, reaching –9.0% and –32% in the tenth generation for the 5% and 15% separation levels. However, the worst animals which were separated out always had higher faecal egg counts compared with the control. This difference increased over generations. For the 10% and 15% worst animals the trajectories of the difference between the control and the separation cases were, in general, different, reducing over time. For the 10% separation case it decreased slowly from 26.3% in the zero

generation to 20.9% in the tenth generation. The difference between the worst animals in the 15% separation case and the control decreased from 20.6% in the zero generation to 3.3% in the tenth generation. The average faecal egg counts of all the animals in the different separation cases was lower from that of the control case. It ranged from approximately –0.5% in the first generation to –6.6% in the tenth generation of the 5% scenario to –17.4% in the tenth generation of the 10% scenario and -26.7% for the tenth generation of the 15% scenario.

When the repeatability of faecal egg counts was 0.6 and selection was for live-weight, separation again had a beneficial effect on the faecal egg counts of the best animals. This difference increased over generations becoming -17.4%, -39.1% and -45.7% in the tenth generation. Combining the two sub-populations resulted in slightly higher faecal egg counts for the separation cases than the control (less than 1%) in the zero generation. However, from the first generation onwards separation has a favourable overall effect on faecal egg counts, with the overall average egg counts being 11.6%, 30.1% and 33.3% lower than those of the control, respectively.

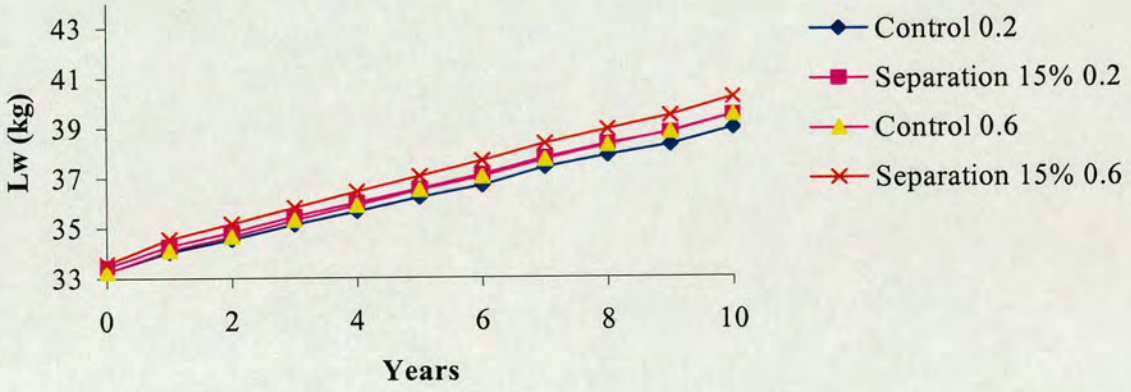

Figure 4.10. Long term response in live-weight when selecting for live-weight. Repeatabilities of faecal egg counts: 0.2 and 0.6. Control and 15% separation level shown for both cases

The direct selection response for live weight is shown in Figure 4.10. When the repeatability of faecal egg counts was 0.2 separation had a positive effect on live-weight, which was slightly higher than the control in the zero generation for all the best animals in all separation cases, of the order of 0.5%. This difference remained constant for the 5% case but increased slowly for the other two cases (10% and 15%) to the of order of 1.2% for the last generation. The animals separated out had lower live weight in all generations than the control, in the first generation, ranging from –4.3% for the 5% separation level to –2.6% for the 15% separation level. There was a small but consistent tendency for this difference to become smaller so that at the tenth generation these differences had decreased to 3.2% and 1.3% for the 5% and the 15% separation case respectively. When averaged across both subpopulations , when separation is practised the animals had slightly higher live weight than the control, but the difference was never greater than 1%.

The difference between the control and the separation cases, for the best animals, followed the same pattern when the repeatability of faecal egg counts was 0.6 as when it was 0.2. However, for the worst animals, the difference from the control case was three times greater and in an unfavourable dirrection compared with the repeatability of 0.2 scenario. The overall effect was that, on average, separation had a small beneficial effect on live-weight. For the 5% separation case this effect was in all generations very close to zero, when when compared with the control, whereas it was slightly higher in the other two scenarios, becoming approximately 1% in the tenth generation.

4.3.2.4 Selection for Live-weight. Carry-over effects

Qualitatively, the results for Fec for this scenario were similar to those for the no carryover effects as can be seen at Figure 4.11. When the repeatability of faecal egg counts was 0.2 the majority of the results for the 5% and 10% were also quantitatively similar to the no carry-

over effect, expressed as percent difference from the control case. For the 15% separation case, however, the results for the best animals were different, in quantitative terms, from the no carryover effects. At the tenth generation the best animals had 41% lower faecal egg count than in the control case compared, with 32% in the no-carryover effect case. This had an effect also on the average faecal egg counts of all the animals: in the carry-over effect case they were 36% lower than the control, whereas in the no carry-over effect they were 27% lower than the control .

When the repeatability of faecal egg counts was 0.6, as in the repeatability of 0.2, the difference between the control and the separation scenarios were qualitatively the same in this scenario as in the no carry-over effects. For the best animals they ranged from slightly different in the 5% separation case to higher in the 15% separation case. The animals which were separated out had higher faecal egg counts but the difference between the separation cases and the control was lower than the equivalent in the no carry-over effects scenario. In the 5% scenario this difference was three quarters the difference of the no carry-over effect while in the other two scenarios it was approximately half. On average the animals in this case had slightly lower faecal egg counts in the 5% and 10% scenarios and substantially lower faecal egg counts in the 15% case compared with the no carry-over effects scenario.

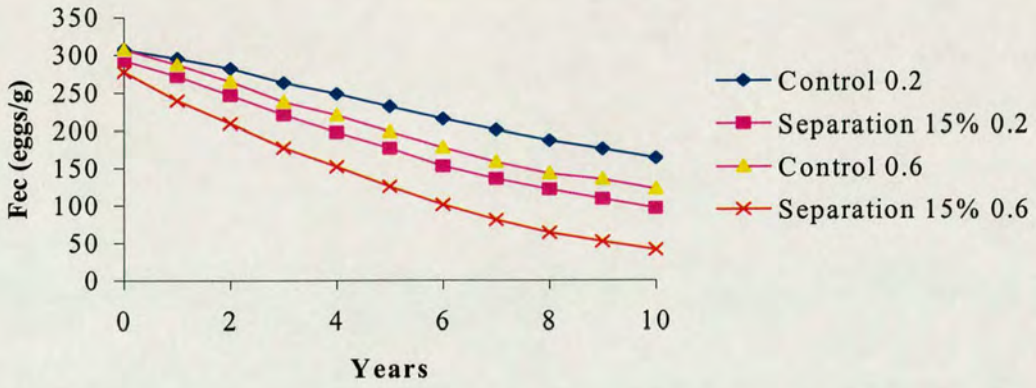

Figure 4.11 Long term correlated response in faecal egg counts when selecting for live-weight. Carryover effects. Repeatabilities of faecal egg counts: 0.2 and 0.6. Control and 15% separation level shown for both cases.

In Figure 4.12 the mean live weights are given for these cases, and the response to selection is seen to be greater than for any of the other scenarios explored in sections 4.3.2.1 – 4.3.2.3. When the repeatability of faecal egg counts was 0.2 the difference between the control and the separation scenarios was higher, and in a favourable direction, compared with the no carry-over effect case. The magnitude of the difference was proportional to the percent of the animals separated. In the 15% separation case the difference between the control and the best animals was 2.9% compared with 1.4% in the no carry-over effects scenario. This was the case also for the animals separated out (also in favourable direction). In the 15% case, the difference between the separated out animals at the tenth generation and the control became even slightly positive (0.5% higher live-weight compared with the control). For the other two scenarios there was a tendency for the difference between the control and the separation cases to become less as selection progressed, but not to the same extent as in the 15% case. The overall result was that the animals in the carry-over effect scenario had, on average, slightly higher live-weight than the control. The highest difference was observed in the 15% separation scenario (2.5%).

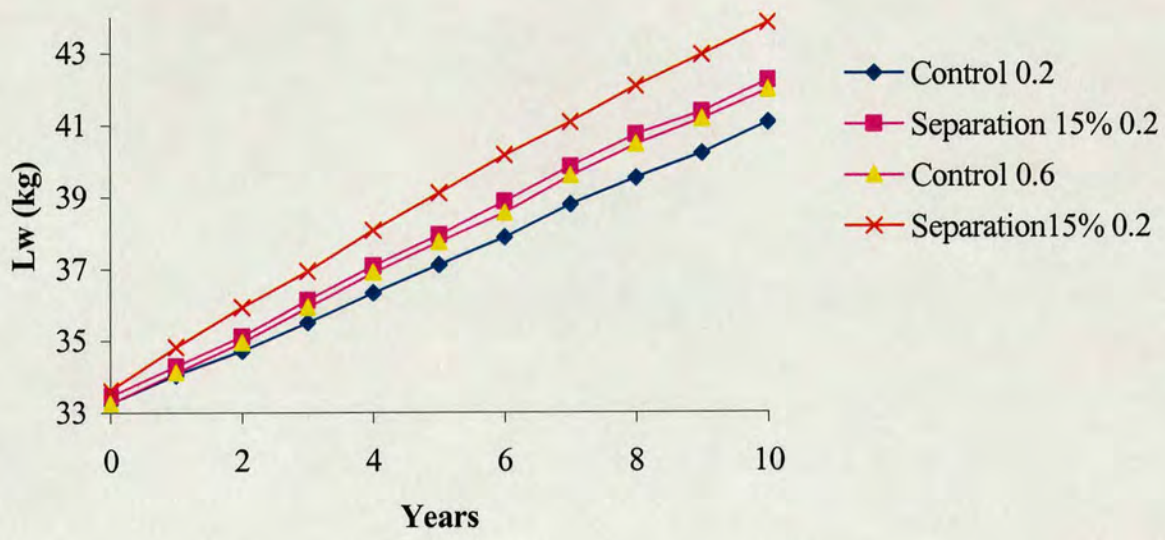

Figure 4.12 Long term response in live-weight when selecting for live-weight. Carryover effects. Repeatabilities of faecal egg counts: 0.2 and 0.6. Control and 15% separation level shown for both cases.

When the repeatability of faecal egg count was 0.6, the same pattern was observed as when the repeatability was 0.2, i.e. the best animals in the carry-over effect had a greater difference from the control than in the no carry-over effects scenario. This difference in the 15% case in the tenth generation was 4.0% in the carry-over effect scenario compared with 1.7% in the no carry-over effects. This was also the case for the animals which were separated out. In this case the animals separated out in the 10% and 15% scenarios had slightly higher live-weight compared with the control (0.9% for the 10% and 1.9% for the 15%). The overall effect was that in the tenth generation the difference between the average of all the animals in the separation scenarios and the control was higher in the carry-over effect compared with the no carry-over effects case. The maximum difference was 4.1% in the tenth generation of the 15% separation (0.97% in the no carry-over effects).

4.3.3 Long term response, when combining culling with selection for either faecal egg count or live-weight. No carryover effects.

In section 4.3.3, results for separation have been considered where the same total number of lambs remain on the field. Now consider the culling scenario, where lambs are physically removed from the pasture and the density of the remaining lambs decrease.

In Table 4.4 the means of the control case for faecal egg counts is given for the 0.2 and 0.6 repeatabilities, at generations four and ten, along with the differences from the control for each culling scenario. The important point emerging from this table is that there are substantial effects from culling compared to separation of lambs and these effects get larger the greater the culling proportion. A large proportion of the difference seen at ten years has already been achieved by the fourth year.

Table 4.4 Effect of different culling levels on faecal egg counts when the selection criterion is either Fec or Lw

R_e of Fec	Selection criterion	Year	Control mean eggs/g	% difference from the control		
				5% cull	10% cull	15% cull
0.2	Fec	4	109	-7.36	-11.7	-17.1
0.2	Fec	10	26	-12.0	-21.3	-28.0
0.6	Fec	4	57	-10.7	-23.5	-33.3
0.6	Fec	10	21	-22.3	-34.9	-46.3
0.2	Lw	4	272	-7.35	-13.5	-21.7
0.2	Lw	10	209	-6.41	-24.0	-31.8
0.6	Lw	4	245	-10.5	-19.9	-28.2
0.6	Lw	10	161	-20.9	-33.8	-51.9

The impact of these culling and selection criteria on liveweight are shown in Table 4.5. In general, when faecal egg count is the selection criterion, the impact of culling upon live-weight is trivial. When selection is on live-weight, the increases in live-weight are greater than in the separation scenario but they are still small.

Table 4.5 Effect of different culling levels on live-weight when the selection criterion is either Fec or Lw

R_e of Fec	Selection criterion	Year	Control mean	% difference from the control		
			kg	5% cull	10% cull	15% cull
0.2	Fec	4	35.5	0.09	0.29	0.55
0.2	Fec	10	36.7	0.64	0.33	0.25
0.6	Fec	4	36.1	0.19	0.14	0.81
0.6	Fec	10	36.7	-0.43	-0.35	0.19
0.2	Lw	4	35.6	0.56	0.84	1.77
0.2	Lw	10	38.9	0.43	1.08	1.90
0.6	Lw	4	35.9	0.49	1.25	1.94
0.6	Lw	10	39.5	0.62	1.28	1.98

4.3.4 Decomposition of the response

In Tables 4.6 to 4.9 the decomposition of the response into its components is given. The results given are for repeatability of faecal egg counts of 0.6 and the separation scenario in a single replicate (out of 20) as a worked example. Results here are on the logarithmic scale, to give a closer approximation to normality, but the rank order of the breeding values of the animals will not change with the transformation.

In all four tables the first column indicates the separation level to which this row refers. In the second column the observed response without culling is given i.e. the response of the control line. In the third column the expected genetic response of the control, as estimated from quantitative genetics theory assuming no environmental change, is given. These predictions are based on estimates of the heritability, phenotypic variances and correlations (wherever appropriate) specific for each separation case and hence the genetic (and subsequently the environmental) response differs between the different scenarios. In the fourth column the environmental response for the control case, estimated as the difference between the observed and the estimated genetic response, is given. In the fifth column the

effect of separation at the base population on the level of the base population is shown. Essentially this is the difference for each trait between the mean for each separation level and that of the control. In the sixth column the effect of separating at the second generation on the same generation is shown assuming separation also in the first generation.

When the selection criterion is faecal egg count, 47% of the response is genetic in origin and 53% environmental as can be seen in Table 4.6 (0.28 *vs.* 0.32). The separation has approximately the same effect, in both generations.

Table 4.6 Decomposing the observed response (log(eggs/g)), when selecting for Fec and separating lambs on the basis of Fec Repeatability of Fec 0.6

	Observed	**Genetic**	**Environmental**	**G0 separation effect on G0**	**G1 separation effect on G1**
5%	0.6	0.28	0.32	0.04	0.06
10%	0.6	0.28	0.32	0.09	0.10
15%	0.6	0.28	0.32	0.13	0.15

As can be seen in Table 4.7, 35% of the observed correlated response of live-weight of the control is due to genetic change. In the second generation the separation effect is almost half of that in the first generation. As the separation level increases the impact of separation becomes higher in both generations.

Table 4.7 Decomposing the correlated observed Lw responses (kg), when selecting for Fec and separating on the basis of Fec. Repeatability of Fec 0.6

	Observed	**Genetic**	**Environmental**	**G0 separation effect on G0**	**G1 separation effect on G1**
5%	1.37	0.48	0.90	0.08	0.04
10%	1.37	0.48	0.89	0.25	0.13
15%	1.37	0.45	0.92	0.37	0.16

In Table 4.8 the decomposition of the response is given for faecal egg count, when the selection criterion is live-weight. The observed response is relatively small, as expected.

55% of the observed response is genetic in origins. The effect of separation in both generations is approximately the same in the two generations for all separation levels.

Table 4.8 Decomposing the observed Fec responses (log(eggs/g), when selecting for Lw and separating on the basis of Fec. Repeatability of Fec 0.6

	Observed	Genetic	Environmental	G0 separation effect on G0	G1 separation effect on G1
5%	0.11	0.06	0.05	0.04	0.05
10%	0.11	0.06	0.05	0.09	0.08
15%	0.11	0.06	0.05	0.13	0.12

In Table 4.9 the decomposition of the response is given for the case when selecting for live-weight. In this case the response for live-weight is somewhat lower that the correlated response, shown in Table 4.7, when selection is for faecal egg counts. However, in this case 69% of the observed response is genetic compared with 35% for live-weight in Table 4.7.

Table 4.9 Decomposing the observed Lw responses (kg), when selecting for Lw and separating on the basis of Fec). Repeatability of Fec 0.6

	Observed	Genetic	Environmental	G0 separation effect on G0	G1 separation effect on G1
5%	1.14	0.79	0.35	0.05	0.08
10%	1.14	0.77	0.37	0.25	0.18
15%	1.14	0.74	0.40	0.37	0.21

4.4 Discussion

4.4.1 Summary

This chapter explores one possible way of exploiting resistance to parasites in order to increase productivity. The base for this approach lies in the fact that faecal egg count has a highly skewed distribution in which few animals harbour the majority of the parasites, functioning therefore as contamination sources. The way envisaged in this chapter for exploiting the distribution of faecal egg counts was a combination of selection and separation/culling. One measurement of faecal egg counts was taken at approximately three months of age and the worst animals were separated or culled on the basis of this measurement. The effect of this practise in combination with selection either for live-weight or faecal egg counts, at six months of age, was examined.

Although there have been studies exploring the distribution of faecal egg count in sheep, there have been no studies examining the practical implications of such a distribution in minimising the problem of gastrointestinal worms. To the author's knowledge there are no data available for the use of the distributional properties of faecal egg count in a way such as the one described in this chapter.

4.4.2 Effect of separation

Generally, separation had only a very small effect on live-weight. The effect was greater when the selection criterion was live-weight rather than faecal egg count and when carryover effects were imposed. The effect was also greater when the repeatability of faecal egg counts was high, i.e. 0.6. The effect on faecal egg count was, generally, in the desired direction (lower faecal egg counts) and greater in magnitude compared with live-weight.

As has been pointed out by Bishop and Stear (1999), there is an asymmetry in the selection response between faecal egg counts and live-weight. Selection for increased productivity will not lead to large improvements in disease resistance. However, selection for resistance to gastrointestinal nematodes gives higher response than predicted by simple quantitative genetics theory. What we observe in our results is that there has to be a large reduction in faecal egg counts to obtain a relatively small increase in live-weight. For example, a reduction of 46% of faecal egg counts for the 15% separation level in the tenth generation of the scenario of selection for live-weight with carry-over effects, results in a 2.9% increase in live-weight when the repeatability of faecal egg counts was 0.2.

The combination of selection for reduced faecal egg counts with separation for the same trait appears not to be beneficial in terms of productivity as measured by live-weight. As shown by Bishop and Stear (1999) selection for reduced faecal egg counts leads to a quick drop of the average faecal egg counts of the flock which subsequently flattens out to a very low value of faecal egg counts. The worm burden of the "wormiest" animals might then be too low to produce a significant production loss and pasture contamination. Thus by separating animals solely on the basis of faecal egg counts one might be losing productive animals, in circumstances in which parasitism has very small effects on production. Therefore it may, indeed, lead to lower live-weight even for the best animals of the flock. On the other hand selection for higher live-weight results in relatively small linear change in faecal egg count (Bishop and Stear, 1999). As the faecal egg counts of the animals in this case are slowly reduced, separation on the basis of faecal egg counts might have a beneficial impact on both traits.

When carry-over effects are combined with separation for faecal egg counts there is a benefit obtained compared with the case of grazing equally contaminated pastures each year, for all

scenarios. In the simulations there were some cases in which the animals that were separated out had even higher live-weight and lower faecal egg counts than the control after several years of selection. When carry-over effects are imposed in the zero generation the animals are separated into two groups, depending on the separation scenario in question. For three months the animals remain separated and at the end of the sixth month their faecal egg counts are measured. This leads to an increase of faecal egg counts for the animals separated out. Next year the new lambs graze the same pasture. The part of the pasture that was grazed by the animals separated out in the previous year, and thus has higher number of infectious larvae larvae, is randomly grazed by all lambs, as there are no restrictions. The same is the case for the remaining part of the pasture which was grazed by the animals with the lower faecal egg count and thus has lower parasitic load compared to pasture grazed by the control animals. The proportion of the animals separated out is lower than the remaining animals and therefore the overall result is that the parasitic load on the whole pasture is lower compared with the pasture grazed by the control animals (no separation). Over years, the cycle is repeated and this lower initial challenge may result in animals separated out having lower faecal egg count than the control. As explained in this discussion this is mainly seen when the selection criterion is live-weight.

The gain from separation however is quite low in all cases and it does not exceed 4%. As pointed out, the distribution of faecal egg count in the simulated data of the first generations was not as skewed as in available field data, where faecal egg count distribution is often highly skewed (Stear *et al.* 1995). Effective implementation of separation/culling depends heavily on the skewness of the distribution of faecal egg counts and lack of skewness will lead to underestimation of the response to separation/culling. However, as selection progresses the distribution of faecal egg count is predicted to become more skewed (Bishop and Stear, 1997). Therefore, the effect of separation/culling in this study is probably an

underestimation, in the first generations but not in later generations. This is especially true for the no carry-over effect situation. In the carry-over effect scenario the effect of separation on previous generations has an impact on the present generation. In this study it was difficult, using the assumptions made, to produce a more skewed distribution in the base generation. For example, the three 'major' components of faecal egg counts, namely: fecundity, mortality and establishment of the parasites, have been assumed to be uncorrelated and with specific distributions. Most probably some sort of unknown correlation exists among these traits and this may result in the very skewed distribution of faecal egg count observed in field data. Moreover, maybe the distribution assumed were incorrect or not variable enough, again contributing to the relative lack of skewness in faecal egg count.

4.4.3 Effect of culling

When culling was practised instead of separation the effect on faecal egg counts was greater. This is due to a within year dilution effect of the faecal egg counts. Culling on the grounds of faecal egg counts, or indeed for any trait, will result on fewer animals grazing a pasture. As a result the density of the infective larvae in the pasture becomes lower. Therefore, the animals face lower levels of larvae challenge and a lower loss of productivity. Other factors, which can have a beneficial impact when culling is practised, like higher available dry matter food intake/animal and improved quality of pasture should not be ignored. It has been shown (Vlassof, 1982) that there is an uneven distribution of infective larvae on the grass, depending on the height of the sward. The majority of the infectious larvae are concentrated on the lower parts of the plants, therefore, taller grass results in lower challenge to the animals. Such effects were not included in this model.

In terms of productivity, culling has a higher effect than separation. However, this effect is compromised by the fact that the farmer will have fewer, albeit heavier animals. For demonstrating this point we will use the case of selection for live-weight with no carryover effects, repeatability of faecal egg counts of 0.6, and separation/culling level of 15%. After ten years of selection and separation the animals have a mean live-weight of 39.9 kg. After ten years of culling the animals have a mean live-weight of 40.3 kg. The control animals have a mean live-weight of 39.5 kg. Therefore, in total the separation practise resulted, on average, in 0.4 kg higher live-weight than the control. On the other hand the culling practise resulted in a 5.27 kg loss of live-weight ($39.5 - 40.3 \times 0.85 = 5.27$). Therefore, there is a trade off which should be taken into account. This calculation, however, does not take into account the value of the culled animals, which may be sold to markets preferring light lambs.

4.4.4 Expectations

There are some reasons why the effect of separation/culling is less than what might be naively expected. The correlation between different faecal egg count measurements is less than one. So, a measurement taken at one day will not rank the animals in the same order as one taken at another time. Therefore, this measurement represents a 'snapshot' of the population at a given point and not a longitudinal picture of the population. As the repeatability increases the 'snapshot' becomes more representative of the whole picture. Density dependence effects were incorporated in the model which essentially represent unwanted 'noise' for the animal breeder. The density dependence effects mean that when there are fewer worms in the gastrointestinal tract they will grow faster and produce relatively more eggs than worms at higher density. The epidemiological effects, in addition to having to be large to influence live-weight, also have a time delay of 2-3 weeks before they start having an effect. This is the time between the actual, physical shedding of the eggs with the faeces and the larvae becoming adult worms in the infected animal.

What this work shows is that there is relatively little advantage in using separation/culling combined with selection, for obtaining additional gain in live-weight in temperate regions. There is probably no great economical gain (if any) from separating or culling the worst animals on the grounds of faecal egg counts, alone. It can possibly be used beneficially in combination with selection, however, for reducing faecal egg counts and altering the disease epidemiology in areas where animals are heavily parasitised.

5. Modeling the joint effects of genotype and protein intake on resistance to gastrointestinal parasite infections in sheep

5.1. Introduction

In earlier chapters the use of genetics to control nematode parasites in grazing ruminants has been considered. Genetic parameters have been estimated for goats where there is a lack of such parameters and for sheep by using a random regression model. Furthermore the exploitation of the distributional properties of faecal egg count combined with long-term selection has been examined *in silico*. In this chapter an attempt is made to quantify the possible nutrition x genotype interaction by means of computer simulation.

As early as 1932 Clunies-Ross and Graham suggested that improved nutritional status of sheep reduces the production losses and mortality rates associated with parasitic nematode infections. Although it has been suggested that nutritional factors influence parasitism, our knowledge of nutrition by parasite resistance interaction is rudimentary (Sykes and Coop, 2001). What is certain though is that dietary protein affects the ability of the host to respond to infection and could be used as a means of minimising the reliance on anthelminthics (Sykes and Coop, 2001).

Improving the resistance of the host to gastrointestinal parasites and dietary protein supplementation has generally been considered as measures implemented separately from one another. However, they are unlikely to be conducted on their own. Most probably they will be implemented, at least for the foreseeable future, along with anthelminthics as a mean of reducing the number of drug treatments per animal per year. Thus the selection pressure for developing anthelminthic resistance in the parasite population will be reduced with the result that drugs will remain effective for a longer period.

From a breeders' point of view protein supplementation might result in the expression of genotype x environment interaction. The ranking of the animals with respect to resistance and productivity may change in different environmental conditions and this would have implications for the design of breeding programs. Additionally, the correlation between resistance and performance might change with dietary protein level.

The interaction of dietary protein supplementation and host genotype is explored in this chapter, under various parasitic challenge scenarios, using computer simulation (Fortran 90). The main focus is the effect of dietary protein on the expression of hosts' performance and resistance, and on the genetic parameters estimates for these traits.

5.2 Materials and methods

5.2.1. Scope of the model

A model was developed which included the impact of protein nutrition level, host-parasite interactions for nematode infections and the development of immunity upon lamb growth. The model was parameterized for a population of hosts at both the genetic and phenotypic level. Output variables included predictions of growth, food and protein intake, parasite burdens and egg outputs for each individual within a genetically structured population, enabling estimation of genetic parameters for each output variable. This model is distinct from that in chapter four which did not allow for variable dietary protein levels nor did it allow causal relationships between parasite burdens, food intake and growth.

5.2.2 Description of the model

5.2.2.1 Single animal

The way in which the nutritional requirements and the growth of lambs were modeled, in the parasite-free case, is shown in the Figure 5.1. Three heritable traits were used for modeling the growth of the lambs: live-weight, food intake and maximum gain. The growth of the animals, in the absence of infection, was modeled so as to fit published tables (NAS, 1985) with respect to protein intake. Values given in these tables made it possible to derive equations relating food intake, live-weight, and protein intake, as explained below. All other nutritional requirements were assumed to be adequately met. Each day, the protein requirement of each animal was estimated based on its live weight.

Food Intake. Expected daily dry matter food intake (FI) was estimated as a quadratic function of live-weight, symbolized by (**A**) in Figure 5.1, derived from tables (NAS, 1985). The **A** function is:

$$FI = (-7.5 \times 10^{-4} \times LW^2) + (7.05 \times 10^{-2} \times LW) - 0.125 \quad \textbf{(A)}$$

where FI is the daily food intake in kg of dry matter and LW is the daily live-weight in kg.

The experimental results of Datta *et al.* (1998) on food intake and growth in parasitised and non-parasitised lambs at differing levels of protein intake, show that the average food intake of the animals, even at the lowest levels of protein intake, increases with time. Therefore, a restriction was imposed so that for the range of protein levels examined the food intake of day *t*, FI_t, was marginally higher than that of day *t-1*, FI_{t-1}. If the FI_t was found to be lower than FI_{t-1}, a small quantity estimated as a function of live-weight was added to FI_t. If needed this procedure was repeated. This purely empirical procedure is shown in Figure 5.1. Without this adjustment the animals on the low protein levels may lose weight, in contrast to the findings of Datta *et al.* (1998). With this adjustment the animals in the low protein levels had food intake and live-weight resembling the ones given by Datta *et al.* (1998). The adjustment should be proportional to the live-weight of the animal so as to take into account the growth status of the animal. The adjustment, when $FI_t \leq FI_{t-1}$ was $FI_t = FI_t + 5\times10^{-4} \times (\frac{LW_t}{20})$.

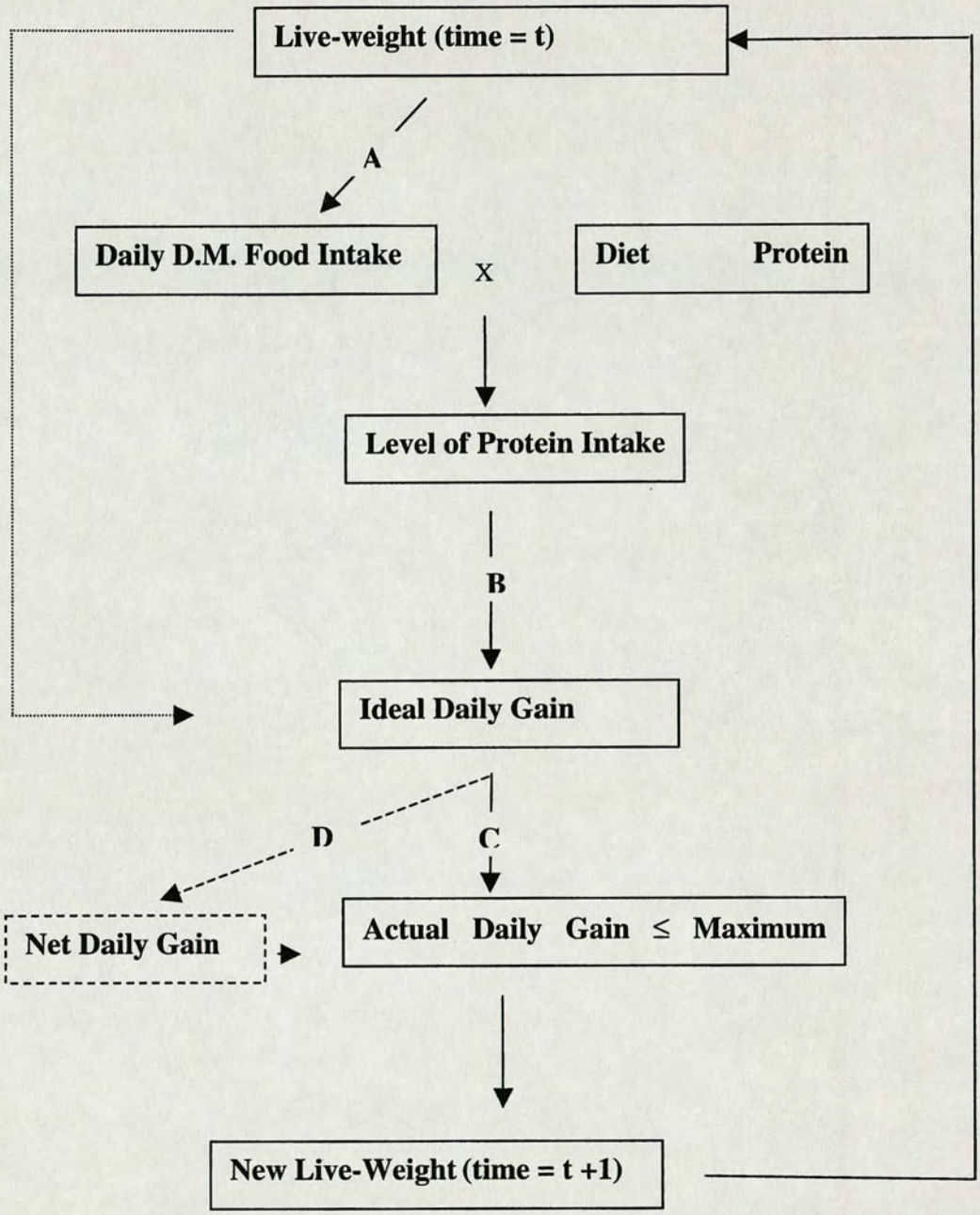

Figure 5.1 Flow chart of the daily growth of the lambs as modeled in the parasite free case. D.M. is dry matter.

Protein intake and requirements. The protein content of the diet expressed as the percent crude protein content of the feed dry matter was set to a predefined level as an input parameter. Using the predicted dry matter food intake and the dietary protein content, protein intake was estimated. From published tables (NAS, 1985) live-weight gain was a linear function of the level of protein intake at any given live-weight. Therefore, live-weight gain ('Ideal Gain' in Figure 5.1) was modeled as a linear function of the level of protein intake at any given live-weight, but the intercept and the slope of the function varied as live-weight changed. For example, the slope and the intercept of the equation connecting protein intake and gain for an animal with live-weight of 30 kg would be different from the slope and the intercept of the equivalent equation for an animal of 20 kg, as shown in Figure 5.2.

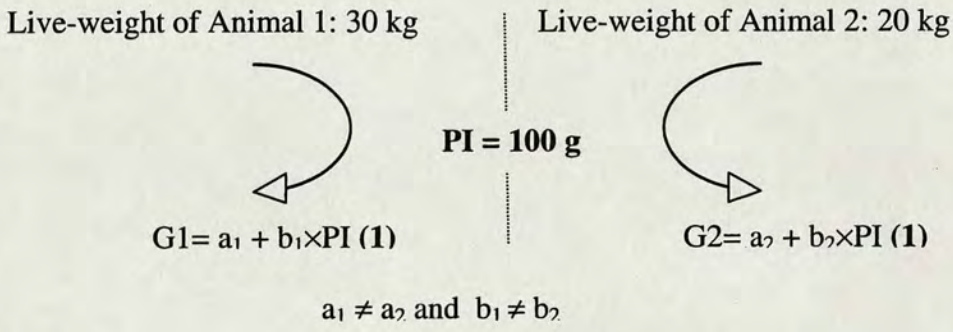

Figure 5.2 Explanation of the different intercept and slopes of the linear function relating protein intake and ideal gain with respect to live-weight. PI is the protein intake, G1 is the gain of animal 1 and G2 is the gain of animal 2

This process is symbolized by (**B**) in Figure 5.1. The function describing the relationship between the intercept in the equations of Figure 5.2 and live-weight is:

$$a_i = 6.73 + 11.4 \times LW$$

where a_i is the intercept and LW is the live-weight.

The equivalent equation connecting the slope of equation (**1**) of Figure 5.2 with live-weight

was derived to be a quadratic:

$$b_i = (7.14\times10^{-4})\times LW^2 + (1.34\times10^{-2})\times LW + 2.24$$

An upper limit to the maximum gain an animal could achieve in ideal conditions as a function of protein intake may be imposed to mimic experimental observations. For example animals in the experiment of Datta *et al.* (1998) were growing at the same rate for 19% or 23% dietary protein content, irrespective of whether they were parasitised or not. Therefore, these authors concluded that the protein needs of the animals would have been satisfied by a diet with 19% protein, irrespective of parasitism status (Datta *et al.* 1998). This similarity in growth rate in their experiment for high levels of protein, suggests that animals were not responding to additional protein and indeed may not have the capacity to grow faster, for the given experimental conditions. That is to say that a lamb of a particular breed and under the specified conditions could with maximum intake of an optimal diet, achieve a certain ("maximum") gain. The animal would not be able to metabolize extra nutrients and they would pass unutilized from the gastrointestinal tract. This is the case, for example, when the physiological satiety has been achieved but not the mechanical one. Therefore the 'ideal' gain was constrained so as not to exceed a predefined maximum gain for each animal. **C** symbolizes this process in Figure 5.1.

Gain. The resultant gain was then added to the live-weight to obtain the new live-weight. The procedure described in Figure 5.1 was then repeated for the number of days examined in this simulation study (from 30 days of age to 180 days of age).

When there was parasitic challenge an extra step was taken in the transition from the 'Ideal Gain' to 'Gain' (Figure. 5.1). This is the pathway **D** in Figure 5.1. which is explained in Figure 5.4.

5.2.2.2 Host-Parasite Model

Host parasite-interactions were based on the model of Bishop and Stear (1997) in which acquired immunity varies between animals and increases with age. The host-parasite interaction is described by three heritable, uncorrelated traits of the host: larval establishment, fecundity of adult female parasites and parasite mortality. Larval establishment represents the proportion of the larvae ingested which survive to become adult parasites. Fecundity is the number of eggs laid by an adult parasite, assuming a constant sex ratio. Mortality is the daily death rate of the adult parasites. Moderate density dependent effects on parasite fecundity were included in the model i.e. as the parasitic load increases the number of eggs produced per worm decreases for a given time period. Therefore, the faecal egg count will increase with worm burden, but not in a linear fashion. The density dependent effects modeled in this study could range from 0 to –1. They were set to –0.25 to represent moderate density dependent effects, i.e. fecundity α worm burden $^{-0.25}$.

When the animals are challenged and infected by larvae, their growth rate is expected to be reduced. Production losses were estimated as a function of larval challenge and worm burden, as shown in part **A** of Figure 5.4 and were in agreement with the results of Datta *et al.* (1998) for the challenged animals (for the period examined by these authors). Worm burden was calculated as the product of worm number and worm size (Bishop and Stear, 1999). For simplicity, the fecundity of the female parasites was used as an indicator trait of worm size, as fecundity is strongly correlated with worm size (Stear *et al.* 1995). The actual penalty values are described below.

A direct link was assumed to exist between the dietary protein content and the ability of the animals to resist the adverse effects of the parasitic infection, and that a higher level of

dietary protein intake will result in lower adverse effects of the parasites (Datta *et al.* 1998, Coop and Kyriazakis 1999). A scaled penalty was applied to the worm burden as a function of dietary protein content with this scaling factor calculated as follows. The 'un-scaled' penalty was applied to the 'average' pasture dietary protein content case, which was assumed to be 14% crude protein. Higher dietary protein contents were assumed to result in a lower penalty and vice versa. The empirical function used to estimate the scaling factor was

$$\text{Scaling} = \frac{\text{DPC}}{\text{APP}}$$

where DPC is the dietary protein content and APP is the average pasture protein. For the range of DPC examined in this study, the value of *Scaling* could be in the range of 0.71 - 1.36 for 10% DPC and 19% DPC respectively. The worm burden was then scaled as a multiplicative function of *Scaling* so that the new quantity (Worm_Burden*) took into account the differences in dietary protein content of the various scenarios. This new quantity was used for estimating faecal egg counts and the penalty is associated with the worm burden (WBP), which is essentially a reduction of growth rate and was estimated using the equation:

$$\text{WBP} = \text{Worm\_Burden}^* \times \text{Worm\_Burden\_Penalty}$$

where the Worm_Burden_Penalty is a scalar and the units of WBP are kg/day.

The penalty placed on the larval intake (LIP) was estimated as a product of the larval intake and a penalty factor. This penalty on the growth rate of the animal worked in the same fashion as WBP for the worm burden, as shown below:

$$\text{LIP} = \text{Larval\_Intake\_Penalty} \times \text{Larval\_Intake}$$

where Larval_Intake_Penalty is a scalar and the units of LIP are kg/day.

Although the penalties remained constant across time, the relative magnitude of these two penalties varied over time. In Figure 5.3, the ratio of WBP to LIP is shown.

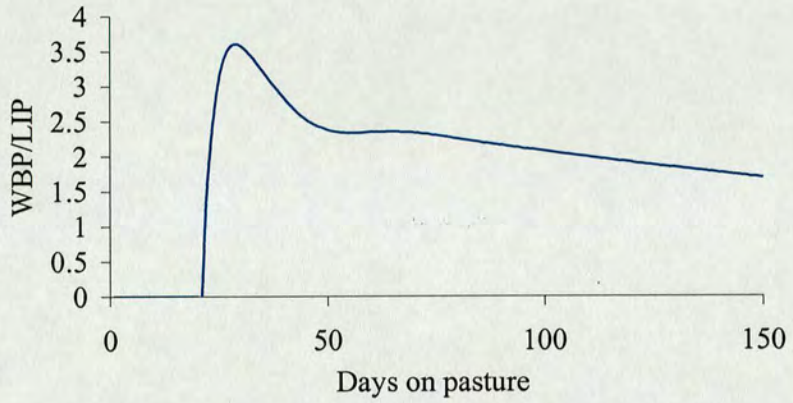

Figure 5.3 Relative magnitude of WBP and LIP expressed as a ratio (WBP/LIP) over time.

The overall penalty imposed (PP) to the daily live-weight gain was estimated as the sum of the two penalties:

$$PP = WBP + LIP$$

The magnitude of this production penalty is equivalent to the one imposed by Bishop and Stear (1999). For the case of animals on an average pasture facing a moderate challenge, the production penalties result in a 30% decrease in growth rate due to parasitism, compared to the control. This production penalty was subtracted from the 'ideal' gain. The resultant 'Net Gain' was then compared against the maximum predefined gain and, if greater than that, constrained. This process is described in part **B** of Figure 5.4 and is the process **D** in Figure 5.1. The new quantity (named 'Gain') is then treated exactly as in the parasite free case: it is added to live-weight.

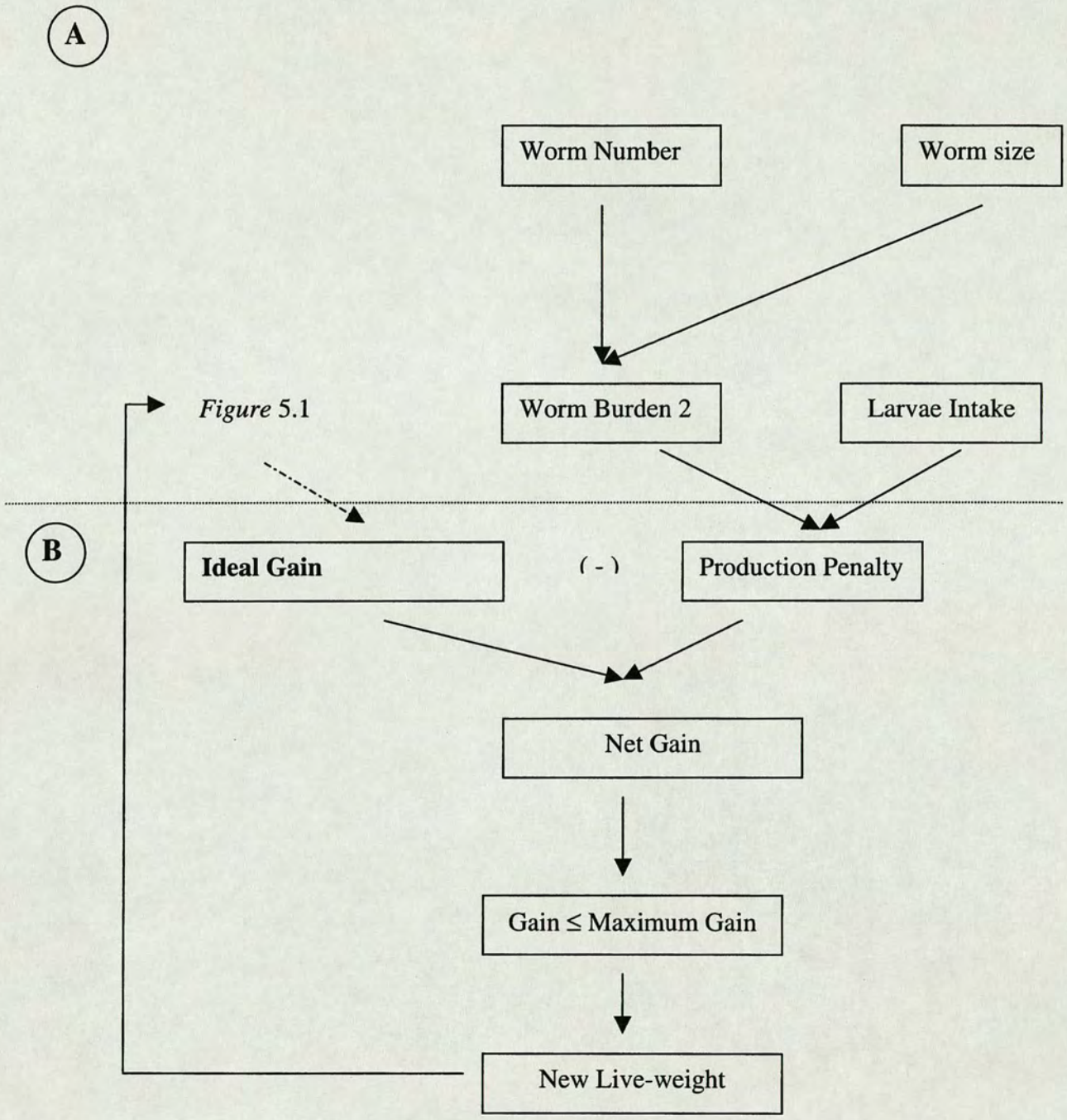

Figure 5.4 Flow – chart representation of the estimation of production penalties (part A) and the imposition of production penalty, due to parasitism, on gain (part B).

5.2.2.3 Parameterizing the growth model at the population level

Thus far, the mechanism of the nutritional part of the model has been explained for a single non-parasitised animal. In this study, however, a population of animals was modeled with genetic relationships between animals, and the variability amongst animals described by genetic and phenotypic parameters. For example, the way that daily food intake has been estimated thus far, would create a phenotypic correlation between food-intake and live-weight of 1. Furthermore, there is no underlying genetic or environmental correlation. Therefore, adjustments have to be made so as the *means* of the traits are according to NAS (1985) but there are genetic and phenotypic relationships between the traits and across time within traits.

Instead of sampling the genetic components of the *lambs* from a normal distribution, these were sampled for the *parents* so as to create a genetic structure in the population, with the appropriate scaling. The animals in the parent generation were assumed to be unrelated. Twenty-five sires were assumed to be mated with five hundred ewes, each sire to twenty ewes. Each ewe produced twins and overall half the lambs were male and half female. These lambs were the population modeled further.

The common environmental and random environmental effects were drawn from normal distributions so that the common environmental component was 0.15 and the heritability 0.3 (coefficient of variation for live-weight 0.1 and for food intake 0.2) in the parasite-free situation both for food intake and live-weight. As the animals were growing the environmental and genetic components of the phenotypes were scaled accordingly. Progeny genotypes were created as the average of parental genotypes plus a Mendelian sampling

term, sampled from a normal distribution with mean of zero and standard deviation of $\sqrt{0.5 \times h^2 \times \sigma_p^2}$

Stochastic variation was introduced so that the observed correlations both between and within-traits across time approximate the expected, and the components of the phenotypic variance of the population should be scaled according to the change of the phenotypic variance. The required genetic and environmental correlation structure, within and between traits, was formed using coefficients obtained from a Cholesky decomposition of the correlation matrix of interest. Using thisit was possible to adjust the correlation both within traits, across time and between traits.

5.2.2.4 Modeling of correlation, within traits

The correlation of live-weights (and food intake), in different time points was modeled using the formula:

$$P_{i,t} = \overline{P}_t + k \times (P^*_{i,t} - \overline{P}_t) + ran(0, \sqrt{(1-k^2)} \times \sigma_t) \qquad (1)$$

Where:

$P_{i,t}$ = value of trait of animal *i* at time *t*.

$P^*_{i,t}$ = mean of trait *after* applying the growth model, prior to adding random variation

$\overline{P}_t$ = mean of trait P^* at time *t*

k = desired correlation between the time points

σ_t = standard deviation of the trait at time *t*.

$ran(0, \sqrt{1-k^2} \times \sigma_t)$ = random number drawn from a normal distribution with a mean of 0 and a standard deviation of $\sqrt{1-k^2} \times \sigma_t$

In this case, the model works on a daily basis. Therefore, we have to add variation *n* times

and the k in equation (1) will be replaced by the n^{th} root of the desired correlation, where n is the number of days for which the flock of animals is simulated. Thus, if the correlation between day 1 and day n is $x \geq 0$, $k_{(t,t+1)} = \sqrt[n]{x}$.

This process can be performed both on the genetic and the environmental components of the phenotype. The components produced after this process can then be scaled by multiplying them with the ratio of $\frac{\sigma_{t+1}}{\sigma_t}$ so that the heritability stays constant.

5.2.2.5 Modeling of correlation, between traits

For modeling the correlations between traits an appropriate form of equation (1) was used. This formula is given below:

$$P_{i,t}^{new} = \overline{P}_t + [(Q_{i,t} - \overline{Q}_t) \times k \times \frac{\sigma_P}{\sigma_Q}] + \left((P_{i,t} - \overline{P}_t) \times \sqrt{(1-k^2)}\right) \qquad (2)$$

where :

$P^{new}_{i,t}$ = new value of trait 1 of animal *i* at time *t*.

$\overline{P}_t$ = mean of trait 1 at time *t*

$\overline{Q}_t$ = mean of trait 2 at time *t*

$P_{i,t}$ = value of trait 1, animal *i* at time *t*

$Q_{i,t}$ = value of trait 2, animal *i* at time *t*

k = desired correlation between the two traits

σ_P = standard deviation of trait 1

σ_Q = standard deviation of trait 2

5.2.2.6 Values of correlations between and across time within traits

In the current study the genetic and environmental correlations were modeled so as to result in the expected phenotypic correlations. The scheme implemented for the correlations is given in Figure 5.5:

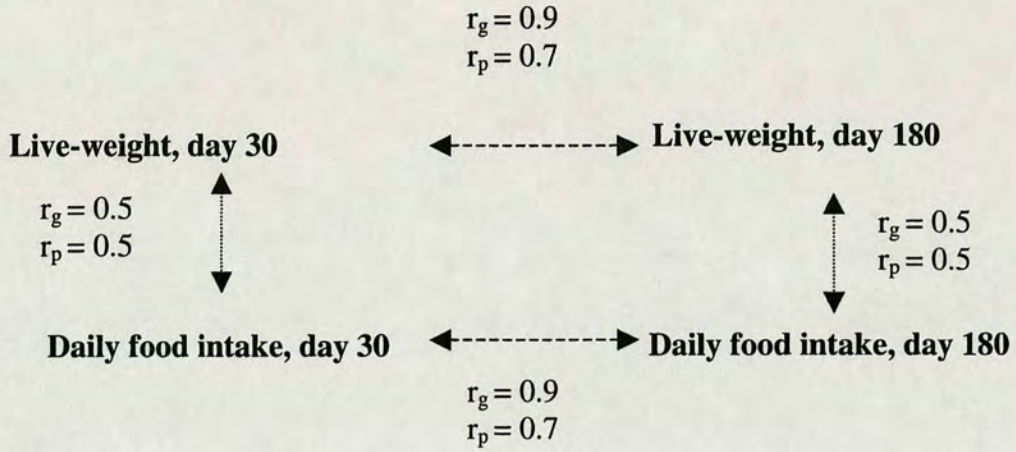

Figure 5.5 Assumed values of correlations across time within traits, and between traits at the same time point used in the present study

The correlation structure of Figure 5.5 was imposed in both the parasite-free and the parasite challenged population so as to give acceptable correlations among and within-traits across time, for live-weight and food intake.

5.2.3 Challenge methodology

5.2.3.1 Parameterization

The model was calibrated by results of Datta *et al.* (1998). Initial mean live-weight was 10 kg and the maximum gain was 220g/day, approximately the same as the 'ideal' growth rate assumed by Bishop and Stear (1999). The animals were assumed to be free of parasites in the first 30 days of their life. Subsequently they were assumed either to be transferred onto an infected pasture where they were naturally challenged or artificially infected with a larval dose. The heritabilities of the parasitological traits were assumed to be 0.2 for establishment and mortality and 0.5 for fecundity. The coefficients of variation for the parasitological traits were: 0.3 for establishment and mortality and 0.4 for fecundity. It should be noted that these values are initial input values and are allowed to change over time, according to the progression of the simulations.

5.2.3.2 Modeling scenarios

In the literature, two methods are used for exploring the relationships between gastrointestinal parasites and the host-animal: natural challenge and artificial challenge (Piper and Barger, 1988). For natural challenge the animals are exposed to parasites through grazing an infected pasture. The parasitic load of this pasture comes from the larvae, some of which survived from the previous grazing season on the pasture and some of which hatched from eggs freshly deposited on the pasture. In the case of the artificial challenge the animals are given a trickle dose of infectious larvae. Usually the animals are individually penned on cages with slatted floor and food is supplied via troughs and thus animals do not face any extra challenge through feeding.

Both artificial and natural challenge scenarios were explored in this study. Artificial challenges were used to initially parameterize the model, and then this was extended to more realistic natural challenge scenario. Three different initial larval pasture contamination levels (for the natural challenge) and larval doses (for the artificial challenge) were explored. In the natural challenge the initial larval challenge of the pasture for each lamb was 'low', 'medium' or 'high'. The 'medium' challenge was calibrated so that the average faecal egg count of the animals resembled the expected average faecal egg count of animals grazing an average pasture. The 'low' challenge level was half the 'medium' challenge and the 'high' challenge three times the challenge of the 'low' challenge. A variation of the natural challenge was explored: the relationship between food intake and faecal egg count was rescaled so that the challenge faced resembled that in the model described by Bishop and Stear (1997). This variation is referred to as high-natural scenario, whereas the original natural scenario is referred as the low-natural. All the initial pasture contamination levels described previously (low, moderate, high) were explored in this case as well. The

relationship was scaled so that the challenge faced by the animals in the high-natural scenario, moderate challenge level, was approximately double the moderate challenge of the low-natural scenario.

In the artificial scenario the same levels of larval dosing per dose as in the natural were assumed. However, the animals were challenged twice per week, at day 3 and day 7 (with the same level of larvae). In this case the larva challenge from the environment, apart from the dosing, was assumed to be zero.

5.2.3.3 Simulation procedure

Twenty replicates were run, each with an initial 1000 lambs for each scenario, in both artificial and natural scenarios. The protein levels used were: 10%, 13%, 14%, 16% and 19% crude protein in the dry matter where the 14% was assumed to represent average pasture protein levels, whereas the other protein levels were the ones used by Datta *et al.* (1998).

The program was run on a daily basis and thus all trait values for each animal were estimated daily. Stochastic variation was introduced daily, in accordance with the genetic parameters and between-trait and across-time correlations (as described above). Animals with live weights outside the range 7-50 kg were removed, i.e. culled or assumed to be sold.

Bivariate analyses, using ASREML (Gilmour *et al.*, 1996), were performed to estimate parameters for output variables such as faecal egg count and productivity. The bivariate analyses were performed separately for each replicate, for faecal egg count vs. live-weight and faecal egg count vs. food intake.

For the unchallenged animals the same procedure was followed as above but with zero challenge (i.e. parasite free situation). Instead of bivariate analyses univariate analyses were performed for live-weight and food intake, using ASREML (Gilmour *et al.*, 1996).

Summary overview

Figure 5.6 shows a very brief summary of the main inputs and the main outputs of the model.

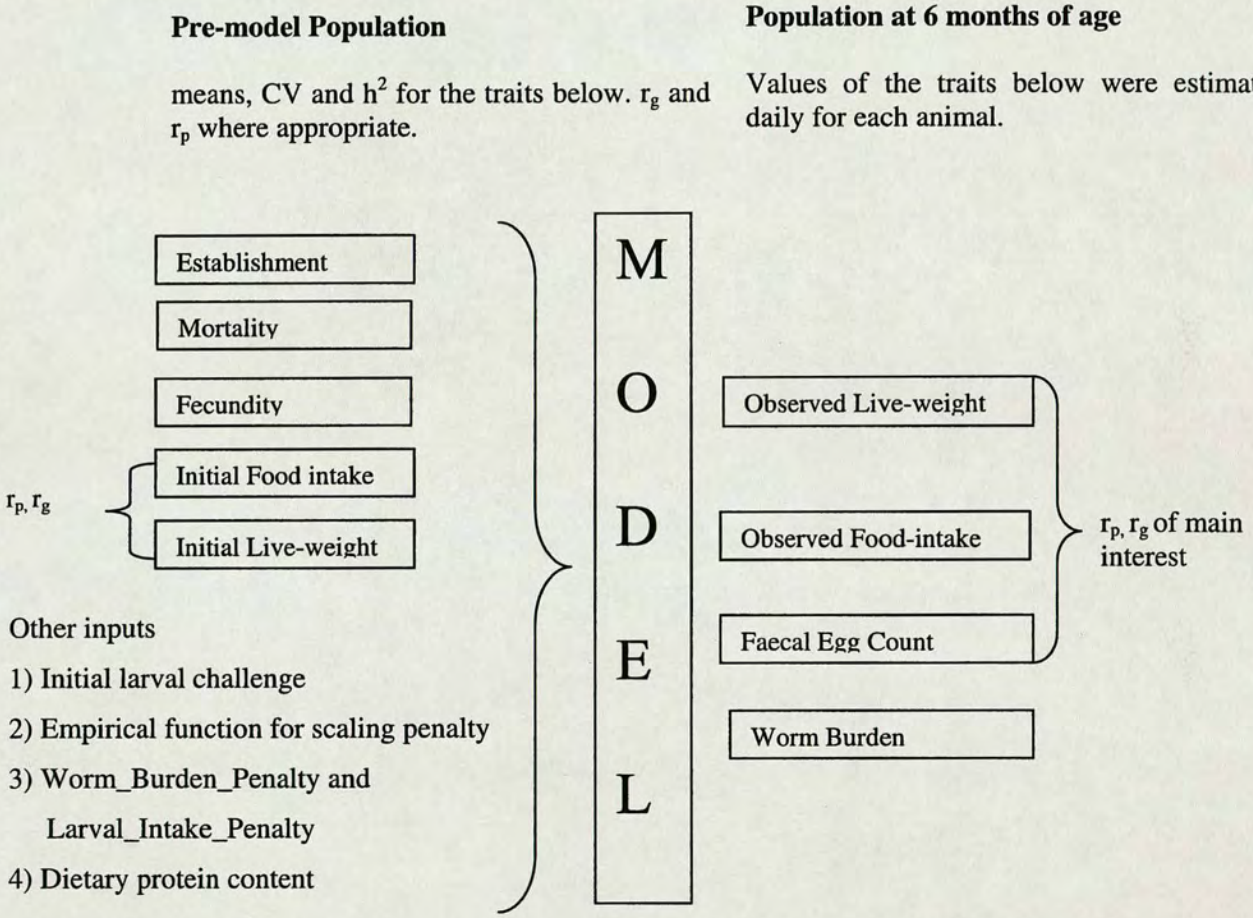

Figure 5.6 Summary of the main features of the model

5.3 Results

5.3.1 Unchallenged animals

5.3.1.1 Means

The means of twenty replicates for live-weight of the animals for the different levels of dietary protein content are shown in Figure 5.7. Live-weight gain increased as the protein plane increases in agreement with expectations and the results of Datta *et al.* (1998). The animals, on the low plane of nutrition (10% dietary protein content), hardly grow in the five months period shown in Figure 5.7. This is also the case for the results of Datta *et al.* (1998) where the lambs grew less than 1.5 kg over the nine-week period studied. Therefore this level of protein intake is extremely inadequate. For this protein level, 36 animals were removed on average at the end of the 6-month period due to having live-weight less than 7 kg. There were usually no animals removed for any of the other dietary protein levels.

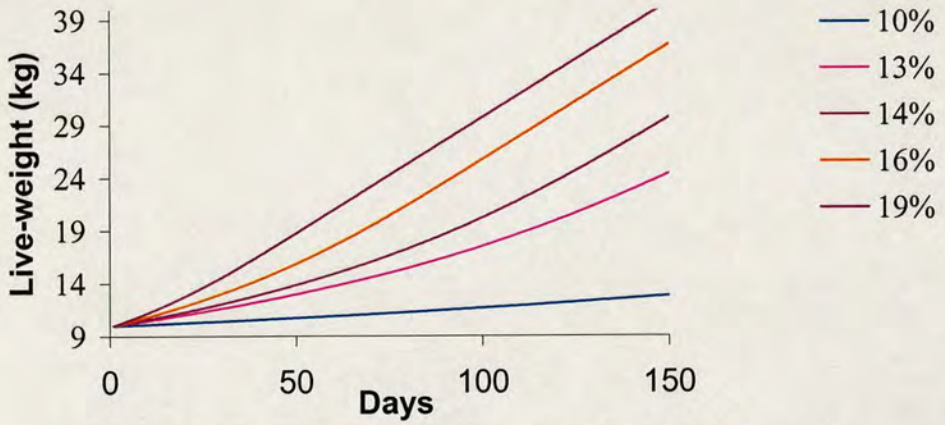

Figure 5.7 Growth rate of unparasitised lambs on diets with different dietary protein contents (%)

In Figure 5.8 the means of the food intake for the unchallenged animals are shown. Higher growth rate and higher food intake are observed as the dietary protein content increases. The general trend is in agreement with Datta et al. (1998). The increasing trend for the 10% protein intake is, partly, a result of the restriction that the food intake on day t should be

higher than the food intake at day t-1. At the end of the time period under examination the gradient of food intake of the animals on the 19% and 16% protein levels is decreasing; this indicates that the food intake of the animals is approaching its limit, which indeed is approximately 1.5 kg, corresponding to the maximum live-weight that can be examined with this model (50 kg).

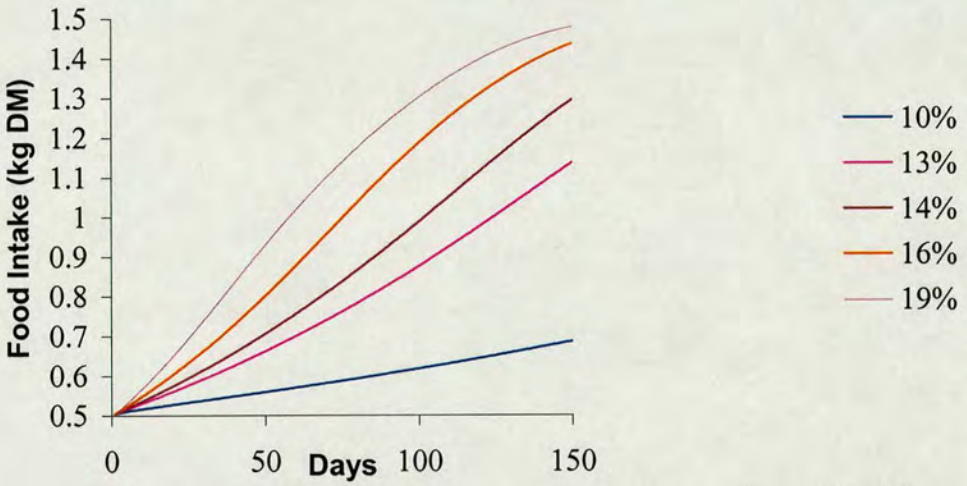

Figure 5.8 Food intake of unparasitised lambs on diets with different dietary protein contents (%).

5.3.1.2. Genetic Parameters

The mean heritabilities of live-weight for the unchallenged animals and of food intake, obtained by averaging the heritabilities of twenty replicates estimated by univariate analysis, are given in Tables 5.1 and 5.2, with their standard errors. They show that the protein level has no significant impact on the heritability of these two traits. The reader is reminded that the assumed heritabilities of 0.3 for live-weight and food intake are starting values. However, the realised output values will change over time as the simulation progresses. The average phenotypic correlation between different dietary protein levels was 0.9 for live-weight and 0.8 for daily food intake.

Table 5.1 Heritability of live-weight, for different levels of dietary protein content

Protein	h^2	s.e
10%	0.17	0.07
13%	0.20	0.07
14%	0.21	0.07
16%	0.21	0.07
19%	0.19	0.07

Table 5.2 Heritability of food intake, for different levels of dietary protein content

Protein	h^2	s.e
10%	0.25	0.08
13%	0.25	0.08
14%	0.26	0.08
16%	0.23	0.08
19%	0.19	0.07

5.3.2 Challenged Animals

5.3.2.1 Challenge Level

An important aspect for comparing the different scenarios with each other, is to know the challenge the animals faced in each scenario. As described in Section 5.2.3.2 an initial arbitrary starting point was initially defined for the naturally challenged animals, with arbitrarily designated low, moderate and high challenge levels. From this point onwards the challenge the animals faced is a function of the larvae on the pasture and the larvae the animals contribute to the pasture and the mortality rate of these larvae. For the artificial challenge case the challenge level was known as the animals were challenged twice per week with the larvae level that had been specified for the naturally infected animals as a starting point (low, medium and high).

In Figure 5.9 the weekly larval intake the animals faced is shown for the three different models at the moderate challenge level and for 14% dietary protein level. These are the artificial scenario, the low-natural and the high-natural scenarios (as described in Section 5.2.3.2).

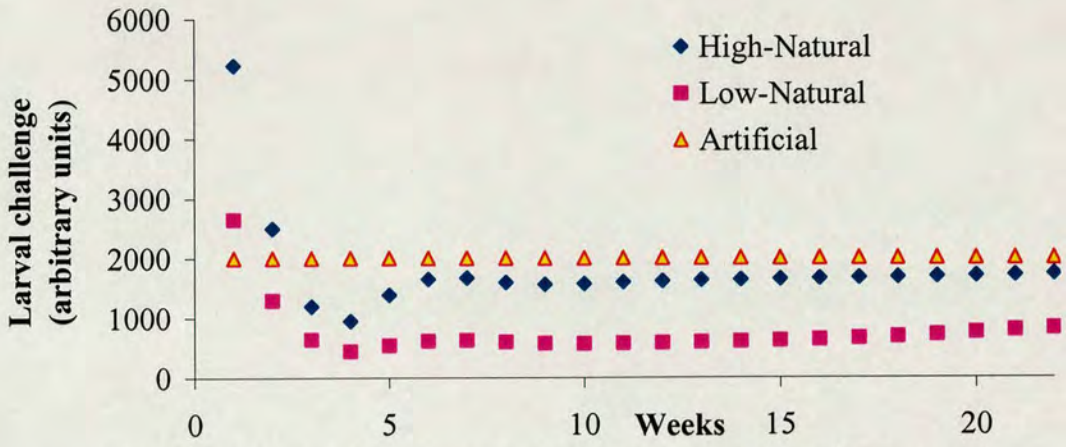

Figure 5.9 Weekly challenge faced by the lambs. 14% dietary protein content, moderate challenge level

In general the challenge faced by the animals in the low-natural scenario is significantly lower than in the other two, except for the first week for which it is higher than the artificial scenario. In the first week the animals in the high-natural scenario face a very high challenge compared with the other two cases. From the third week onwards the challenge faced by these animals is lower, but quite close to that faced by the artificially challenged animals. It reaches a minimum on week four and then it increases sharply in weeks five and six after which the challenge faced by the animals increases only marginally with time. This is also the general pattern in the low-natural scenario.

5.3.2.2 Animals Removed

Table 5.3 shows the number of animals that were removed for each dietary protein content and infection level, for all scenarios. The number of animals removed was higher for the high-natural scenario, lower for the artificial challenge and even lower for the low-natural scenario, reflecting the challenge that the animals faced. It seems that for the animals in the high-natural scenario, the very high challenge faced by them in the first two weeks has a

carryover effect in their later life. In all the scenarios quite a large number of animals were removed from the flock for the 10% dietary protein content level, especially when the parasitic challenge was high. Because this was the case, the results for this dietary protein content should be treated cautiously, especially when the larval challenge is high. As expected the number of animals removed becomes lower as protein level increases and parasitic challenge decreases.

Table 5.3 Average animals removed (out of 1000) due to Lw being less than 7 kg per scenario modeled

	10%			13%			14%			16%			19%		
	NH	NL	A	NH	NL	A	NH	NL	A	NH	NL	A	NH	NL	A
High	*	617	882	880	40	173	338	7	70	28	0	3	1	0	3
Medium	*	411	651	398	11	39	107	2	11	3	0	0	0	0	0
Low	*	231	238	76	3	6	16	0	1	0	0	0	0	0	0

Larval challenge level in rows. Dietary protein content in columns blocks. NH= high-natural scenario, NL=low-natural scenario, A= artificial scenario. For NH and 10% protein almost all animals were removed during test runs. Thus this scenario was not explored. * Signifies that too many animals were removed for the data to be useful.

Animals heavier than 50 kg, were removed only on the 19% dietary protein intake for both artificial and natural challenge. The number of animals removed were exactly the same in both artificial and low-natural scenarios: 6 for low, 3 for medium and 1 for high challenge. Although the number of the animals removed were the same in both challenge scenarios, different animals were removed.

5.3.2.3 Properties of output traits

In Table 5.4 the standard deviations of live-weight and food intake are given, for moderate challenge level and all models. Along with these, the standard deviation for the above traits of the unchallenged animals (refered to as the control) are shown for comparison.

Table 5.4 Standard deviations for Lw and FI, moderate challenge level and the control

Diet protein content	Live-weight (kg)				Daily food intake (kg DM/day)			
	Control	Low	High	Artificial	Control	Low	High	Artificial
10%	2.50	1.26	*	1.24	0.11	0.06	*	0.06
13%	3.62	3.06	1.66	3.30	0.12	0.13	0.07	0.14
14%	3.68	3.49	2.53	3.99	0.10	0.14	0.10	0.16
16%	3.74	3.70	4.03	4.19	0.05	0.08	0.14	0.10
19%	3.78	3.79	3.87	3.97	0.03	0.04	0.05	0.04

In general the magnitude of the standard deviations is the same in all cases although the standard deviations tend to differ more from the unchallenged animals at the 10% protein level, both for live-weight and for food intake. This could be a consequence of the lower limit imposed on the live-weight (7 kg).

In Table 5.5 the percentage reduction of live-weight is shown for all the challenge scenarios. The final live-weight of the control is also shown in this table.

Table 5.5 Percent reduction[1] of Lw for different challenge scenarios at six months of age, for different infection levels and dietary protein content.

Diet Protein Content	Control (kg)	Artificial Scenario			Natural Scenario Low			Natural Scenario High		
		L	M	H	L	M	H	L	M	H
10%	12.8	23	36	41	25	34	38	*	*	*
13%	24.4	24	39	51	25	36	46	47	63	69
14%	29.8	20	36	49	20	32	41	42	59	68
16%	36.7	7	17	28	7	14	22	16	36	54
19%	40.7	2	4	7	2	4	7	4	10	17

[1] Estimated as : 100 x (control - Y) / control

Table 5.5 shows that the live-weight increases as the protein level increases and as the level of infection decreases, as expected. The difference between the live-weight of the animals on the 10% and 19% dietary protein levels is approximately 30 kg. The 10% protein level is inadequate and many animals had to be removed. At the low-natural scenario some animals

exceeded the 50 kg (upper threshold of the model) and were also removed. Therefore, their live-weight is not included in the estimation of these means and subsequently the means presented are an underestimation of the true 'whole flock' means as no lambs were removed due to low live-weight. As an example, in the moderate parasitic challenge and 19% dietary protein content the impact of the animals removed, assuming that from the day of their removal they would have been growing on 0.2 kg/day, would be to increase the mean by 0.3 kg. The greatest reduction is observed in the high-natural scenario.

Table 5.6 Percent reduction[1] of FI for different challenge scenarios, infection levels and diet protein content. FI of the control in kg DM/day.

Diet Protein Content	Control (kg DM/day)	Artificial Scenario			Natural Scenario Low			Natural Scenario High		
		L	M	H	L	M	H	L	M	H
10%	0.69	13	13	11	16	17	16	*	*	*
13%	1.14	19	32	41	20	30	39	38	48	46
14%	1.30	14	27	39	14	24	32	32	49	55
16%	1.44	3	9	17	3	7	12	8	23	41
19%	1.48	0	1	2	0	1	2	1	3	6

[1] Estimated as : 100 x (control - Y) / control

In Table 5.6 equivalent values for the reduction in food intake are given. Food intake follows the same trend as live-weight, being low for low levels of protein and high for high levels of dietary protein content. The absolute differences between artificial and challenge scenarios are smaller than for live-weight.

Table 5.7 Means of Fec (eggs/g) for different challenge scenarios, infection levels and diet protein content.

Diet Protein Content	Artificial Scenario			Natural Scenario Low			Natural Scenario High		
	L	M	H	L	M	H	L	M	H
10%	547	900	*	485	640	750	*	*	*
13%	277	555	862	239	311	368	456	596	677
14%	211	425	691	195	251	291	378	498	586
16%	144	258	390	156	191	212	271	356	420
19%	114	193	264	122	153	173	177	234	275

In Table 5.7 the faecal egg counts, on the last day, are shown for the artificial and the natural challenge scenarios The faecal egg count is, generally, higher for the high-natural scenario case because the animals are constantly challenged with high levels of larvae compared with the low- natural scenario. However, comparing the high-natural scenario with the artificial, the infection level that the animals face is sometimes higher in the natural case, this is the case certainly for the high-natural scenario.

A point to notice is the pattern of the Fec, with respect to protein level, in the artificial and high-natural scenario, high challenge. For the 13% and 14% diet protein content the animals in the artificial scenario have substantially higher Fec. However, this is not the case for the 16% and the 19% diet protein content where the animals in the natural high scenario have slightly higher Fec. This pattern could be the result of an interaction between diet protein content and level and pattern of challenge. In the artificial challenge scenario the animals are challenged twice per with a high dose of larvae and are on poor diet. In the high-natural scenario, there is an initial high challenge, which subsequently diminishes and stabilises to a lower challenge compared with the artificial scenario, as shown in Figure 5.7. In contrast it seems that the 16% and 19% the animals seem to consume levels of protein which mask these differences.

5.3.2.4 Genetic and Phenotypic Parameters

5.3.2.4.1 Bivariate analyses of faecal egg count and live-weight

In Table 5.8 the results of the bivariate analysis of faecal egg count and live-weight at 6 months of age (as for all the bivariate analyses), for artificial challenge are given. The heritability estimates were similar across all protein levels and were similar for the different challenge levels. Therefore differences in protein diet content and in challenge levels do not have an impact on the estimate of heritability, in these simulations.

Table 5.8 Results of the bivariate analysis of Fec vs. Lw for the artificially challenged animals

Diet Protein Content	h^2		r_g			r_p		
	Fec	Lw	L[1]	M	H	L	M	H
10%	0.34	0.14	-0.20	-0.16	*[2]	-0.12	-0.06	*[2]
13%	0.32	0.20	**-0.12**[3]	**-0.22**	-0.25	**-0.15**	**-0.15**	-0.14
14%	0.35	0.20	**-0.14**	**-0.28**	-0.22	**-0.11**	**-0.15**	-0.15
16%	0.35	0.21	**-0.07**	**-0.07**	**-0.15**	**-0.05**	**-0.07**	**-0.11**
19%	0.33	0.21	**-0.06**	**-0.02**	**-0.07**	**-0.03**	**-0.02**	**-0.03**
s.e.	0.02	0.02	0.05	0.05	0.05	0.01	0.01	0.01

[1] L= low level challenge, M= medium level challenge, H= High level challenge
[2] Very few replicates converged
[3] Bold indicates that less than 1/20 of the animals removed by day 180

The correlations between faecal egg count and live-weight were always negative. The correlations for the animals on the 10% dietary protein level were not readily comparable with the rest since many animals were removed (see previous tables), and results for the high challenge have been omitted since most of the animals had been removed. As shown in Table 5.3, a large number of animals were removed for other combinations of dietary protein content and level of challenge. As an indicator, the correlations that were estimated from populations with at least 95% of the initial animals are shown in bold. As dietary protein content increases, the correlations change from slightly negative towards zero. The estimates

for the genetic correlations are slightly stronger for the moderate and the high challenge levels compared with the low infection challenge levels. The parameters estimated for the moderate and the high infection levels are similar.

In Table 5.9 the results for the bivariate analysis between faecal egg count and live-weight are shown for the high-natural challenge. As previously, the heritabilities for different dietary protein levels are quite similar, although slightly lower compared to those for artificial challenge. The correlations follow the same pattern as in the artificial challenge but they are lower. It can be seen that the correlations in bold are very low and the genetic correlations are usually not significantly different from zero.

Table 5.9 Results of the bivariate analysis of Fec vs. Lw for the naturally challenged animals. High - natural scenario.

Diet Protein Content	h^2		r_g			r_p		
	Fec	LW	L[1]	M	H	L	M	H
13%	0.31	0.15	-0.09	0.02	-0.22	-0.05	-0.04	0.02
14%	0.31	0.17	**-0.04**[2]	-0.08	-0.03	**-0.04**	-0.02	-0.02
16%	0.35	0.21	**-0.02**	**-0.03**	**-0.15**	**0.00**	**-0.03**	**-0.05**
19%	0.33	0.21	**-0.04**	**-0.01**	**-0.04**	**0.03**	**0.03**	**0.01**
s.e.	0.02	0.02	0.05	0.05	0.05	0.01	0.01	0.01

[1] L= low level challenge, M= medium level challenge, H= High level challenge
[2] Bold indicates that the animals removed by day 180 were less than 1/20 of the initial population

The results of the bivariate analysis of faecal egg count and live-weight for the low-natural challenge are shown in Table 5.10. The heritability estimates are similar in magnitude to those in tables 5.8 and 5.9. The correlations are similar to those in the high-natural challenge and the genetic correlations are generally not significantly different from zero.

Table 5.10 Results of the bivariate analysis of Fec vs. Lw for the naturally challenged animals. Low-natural scenario.

Diet Protein Content	h^2		r_g			r_p		
	Fec	FI	L[1]	M	H	L	M	H
10%	0.32	0.14	-0.03	0.07	-0.05	-0.03	-0.01	-0.04
13%	0.32	0.19	**-0.10**[2]	**-0.08**	**-0.02**	**-0.02**	**-0.05**	**-0.04**
14%	0.33	0.20	**-0.01**	**-0.11**	**-0.10**	**0.00**	**-0.02**	**-0.04**
16%	0.34	0.20	**0.00**	**-0.01**	**-0.04**	**0.02**	**0.01**	**0.02**
19%	0.32	0.21	**0.03**	**-0.02**	**-0.02**	**0.02**	**0.02**	**0.03**
s.e.	0.02	0.02	0.05	0.05	0.05	0.01	0.01	0.01

[1] L= low level challenge, M= medium level challenge, H= High level challenge
[2] Bold indicates that the animals removed by day 180 were less than 1/20 of the initial population

5.3.2.4.2 Bivariate analyses of faecal egg count vs. food intake

The estimated parameters from the bivariate analysis of faecal egg count and food intake at six months of age for the artificial challenge are shown in Table 5.11. The heritability estimates for faecal egg count are similar to those obtained by the equivalent bivariate analysis of faecal egg count and live-weight. The heritability estimates for food intake are similar to the ones obtained for live-weight. However, the correlation estimates are generally larger negative values than those obtained from the bivariate analysis of faecal egg count with live-weight particularly for the low diet protein contents. In general the pattern of the correlations is similar to those obtained for the bivariate analysis of faecal egg count and live-weight. The correlations become stronger as the dietary protein content decreases and the challenge level becomes higher although at the 19% protein level the estimates are quite similar across challenge levels.

Table 5.11 Results of the bivariate analysis of Fec vs. FI for the artificially challenged animals

Diet Protein Content	h^2		r_g			r_p		
	Fec	FI	L[1]	M	H	L	M	H
10%	0.35	0.28	-0.21	-0.20	*[2]	-0.24	-0.19	*[2]
13%	0.32	0.22	**-0.24**[3]	**-0.30**	-0.26	**-0.32**	**-0.32**	-0.29
14%	0.35	0.21	**-0.18**	**-0.33**	-0.24	**-0.24**	**-0.32**	-0.34
16%	0.35	0.22	**-0.07**	**-0.09**	**-0.19**	**-0.10**	**-0.16**	**-0.23**
19%	0.32	0.19	**-0.01**	**0.02**	**-0.02**	**-0.04**	**-0.06**	**-0.05**
s.e.	0.02	0.02	0.05	0.05	0.05	0.01	0.01	0.01

[1] L= low level challenge, M= medium level challenge, H= High level challenge
[2] very few replicates converged
[3] Bold indicates that the animals removed by day 180 were less than 1/20 of the initial population

Table 5.12 Results of the bivariate analysis of Fec vs. FI for the naturally challenged animals. High - natural scenario.

Diet Protein Content	h^2		r_g			r_p		
	Fec	FI	L[1]	M	H	L	M	H
13%	0.31	0.25	-0.12	0.00	0.33	-0.10	-0.04	0.15
14%	0.31	0.23	**-0.06**[2]	-0.08	0.01	**-0.10**	-0.08	-0.06
16%	0.35	0.25	**0.00**	**-0.12**	**-0.10**	**-0.03**	**-0.10**	**-0.13**
19%	0.32	0.21	**-0.02**	**0.01**	**-0.04**	**0.02**	**0.02**	**-0.01**
s.e.	0.02	0.02	0.05	0.05	0.05	0.01	0.01	0.01

[1] L= low level challenge, M= medium level challenge, H= High level challenge
[2] Bold indicates that the animals removed by day 180 were less than 1/20 of the initial population

In Table 5.12 the results of the bivariate analysis of faecal egg count and food intake are shown for the high-natural challenge scenario. As many animals were removed in this case not all correlations are meaningful. The correlations in bold are to similar those obtained by the bivariate analyses of faecal egg count and live-weight. Again the genetic correlations are generally not significantly different from zero.

In Table 5.13 the results of the bivariate analysis of faecal egg count and food intake are shown for the low-natural challenge scenario. The heritabilities are again largely unaffected

by the level of the parasitic challenge. The correlations are generally even lower than for the high-natural challenge.

Table 5.13 Results of the bivariate analysis of Fec vs. FI for the naturally challenged animals. Low-natural scenario.

Diet Protein Content	h^2		r_g			r_p		
	FEC	FI	L[1]	M	H	L	M	H
10%	0.32	0.28	-0.06	-0.06	-0.10	-0.07	-0.07	-0.05
13%	0.32	0.23	**-0.04**[2]	**-0.10**	**-0.11**	**-0.08**	**-0.11**	**-0.12**
14%	0.33	0.25	**0.01**	**-0.04**	**-0.07**	**-0.05**	**-0.07**	**-0.09**
16%	0.34	0.25	**0.02**	**0.00**	**-0.03**	**0.02**	**0.01**	**-0.01**
19%	0.32	0.20	**0.02**	**0.00**	**0.02**	**0.02**	**0.03**	**0.04**
s.e.	0.02	0.02	0.05	0.05	0.05	0.01	0.01	0.01

[1] L= low level challenge, M= medium level challenge, H= High level challenge

[2] Bold indicates that the animals removed by day 180 were less than 1/20 of the initial population

5.4 Discussion

5.4.1 Nature of the model

This chapter has provided a general framework for assessing the nutrition × genotype interaction for lambs naturally or artificially infected with gastro-intestinal nematode parasites. The growth of lambs was modeled on a daily basis, with respect to protein intake. All other nutrient requirement were assumed to be sufficiently met by the daily food intake of the animal. The host parasite interaction was based on the model of Bishop and Stear (1999). Infection from parasites was assumed to have a cost in the utilisation of protein which in turn affects the growth rate of the animal. The growth rate penalty had two components: one on larval intake and one on worm burden. The penalty based on the worm burden was scaled using an empirical relationship so that high levels of dietary protein resulted in a lower penalty.

The levels of dietary protein content examined were 10%-19% crude protein with 14% assumed to be an average pasture level. The level of artificial infection was assumed to range from moderate to high with the lambs challenged twice each week. In the natural infection scenario the pasture was assumed to provide an initial challenge on pasture of a similar magnitude as the artificial challenge and subsequently the larval intake of the lambs was a function of the contamination of the pasture due to the shedding of parasite eggs in their faeces.

The covariance between faecal egg counts and the production traits was not modeled explicitly but were created implicitly through the relationship of production traits and worm burden to protein intake, the relationship of egg output to faecal output and hence food intake, and the production penalty imposed due to worm burden and larval intake.

The results for live-weight for different dietary protein intakes fit well the published data used to parameterise the model (Datta *et al.* 1998). The growth of the animals was extremely inadequate for the 10% dietary protein content and even when unaffected, they hardly grew. Mortality due to other factors not related to faecal egg count was not modeled. However, in a real life situation the animals would have been severely under-nourished and would be an easily infected from other diseases. Therefore, the growth of the animals shown in Figure 5.7 for the 10% diet protein intake may not be unduly pessimistic.

The effect of nutritional, and in the majority of cases protein, supplementation as a mean of overcoming the effects of gastrointestinal parasitism of sheep has been described in several studies. An overview of the interaction between nutrition and gastrointestinal parasitism is given by Sykes and Coop (2001) who concluded that 'protein supply undoubtedly affects the ability of the host to respond to infection'. Thus, modeling the impact of nutrition on resistance as we have done seems reasonable and justified.

A conceptual framework was developed by Coop and Kyriazakis (1999), who based on the current state of knowledge available in the literature proposed a description for the utilisation of limited nutrients, and more importantly protein, by animals infected with gastrointestinal parasites. They divide the growth of the lambs into two parts: the acquisition phase of immunity and the expression phase of immunity, which are not mutually exclusive. This is summarised in Table 5.14.

Table 5.14 A possible ordering of the priorities (1 highest to 4 lowest) given by a growing animal to its various functions when partitioning a scarce food resource[a].

Acquisition Phase	Expression Phase
1. Maintenance of body protein[b]	1. Maintenance of body protein [b]
2. *Acquisition of immunity*	2.Protein gain
3. Protein gain	3.*Expression of immunity*
4. Maintenance and gain of body lipid	4.Maintanance and gain of body lipid

[a] For a naive, growing animal without prior experience to a challenge the phase of acquisition of immunity is considered seperately from that of expression of immunity.
[b] This includes repair, replacement and reaction to damaged or lost tissue.

From: Coop and Kyriazakis (1999)

The partitioning framework of Coop and Kyriazakis (1999) proposes a way of prioritising the use of protein (in this case) that is scarce and does not sufficiently cover the needs of an animal. Modeling the framework would require data that are not available, for example: what proportion of protein should be allocated to each function and how should the transition from the one phase to the other be modeled? In short, their framework may be conceptually attractive but its implementation would involve many assumptions and require data that are not available. However, some components proposed by Coop and Kyriazakis (1999) are implemented in the current model. The inherited immunological traits of the host, namely fecundity, establishment and mortality of the parasites, change over time so as to mimic the development of the immunocompetence of the lambs. This is essentially equivalent to transitioning from the phase of acquisition to the phase of expression of immunity in the framework of Coop and Kyriazakis (1999). Additionally, in the current model the maintenance of protein is prioritised over protein gain and expression of immunity, which are given equal priority, however lipid dynamics are ignored.

5.4.2 Model outputs

The growth of an animal depends on: a) the protein level consumed, b) the level of challenge, and c) the challenge scenario. The challenge scenario (artificial or natural) is purely managemental. The crucial difference between the challenge scenarios, is that the natural challenge level is a function of food intake and subsequently of live-weight, whereas in the artificial challenge level is not a function of production traits. The animals will normally be infected naturally and would be expected to perform under natural challenge. The artificial scenario might be informative from a parasitologists perspective, however, since more parameters of the system host-parasite are under the experimenters' control. In the natural infection case the developing immune response of the animals contributes to the lower re-infection of the pasture and subsequently lower challenge of the animals.

The natural challenge seemed to result in greater growth reduction than artificial challenge mainly as a consequence of the very high initial challenge, which penalizes initial lamb growth. The high initial challenge in the natural challenge is, partly, a result of the parameterization of the model. A different set of parameters for the three immunological traits of the host and for larval mortality on the pasture could result in model requiring a lower initial challenge. However, the pattern of faecal egg count and worm burden in this study is similar to that predicted and observed in the extensive modeling study of Beecham J.A., Coop R.L. and Jackson. F (Unpublished manuscript).

Studies of the genetic parameters of faecal egg counts and production traits have been performed in various countries including New Zealand (Morris *et al.* 2000), Australia (Eady *et al.* 1998), UK (Bishop *et al.* 1996), Poland (Charon *et al.* 1999) and Kenya (Baker 1998). Many parameters in these studies differed but the heritabilities had generally the same magnitude: 0.2-0.4 (Bishop and Stear 1999). In this study two of the input parameters

involved varied: the level of the challenge and the level of the dietary protein intake for each challenge scenario. The heritabilities of all the traits were unaffected by the level of nutrition and they were in agreement with published results.

The artificial challenge scenario gave larger effects than the natural challenge, in terms of the influence of protein % and challenge level on correlations between traits, which are more apparent for the correlation estimated between faecal egg count and food intake than those between faecal egg count and live-weight. In these cases, correlations become stronger as dietary protein level is reduced and the challenge is increased. There is little discernible pattern for natural challenge. The magnitude and the pattern of the correlations differ from those estimated by Bishop and Stear (1999) who estimated genetic correlations ranging from –0.16 for a production penalty equivalent to a quarter of the 'benchmark' penalty to –0.40 for a quadruple of the 'benchmark' penalty.

There appear to be two published studies examining the genotype by nutrition interaction. In a study involving one relatively resistant breed (Scottish Blackface) and one relatively susceptible breed (Finn Dorset), Abbot *et al.* (1985) found that supplementation with protein had an effect on reducing the faecal egg count of the susceptible genotypes (Finn Dorset) but not of the resistant genotypes (Scottish Blackface). A similar result was obtained by Gray (1997) where the reduction in faecal egg count was much more apparent in random bred protein-supplemented Merinos compared with resistant Merinos. This reduction of faecal egg counts would have the effect of 'weakening' the correlations as the protein level increased as observed in this study. In all challenge methods the correlations estimated for the 19% dietary protein level are lower than those estimated for lower dietary protein levels. This is more apparent in the artificial challenge, which is the closest scenario to that explored by Abbot *et al.* (1985) and Gray (1997).

The results for the correlations were perhaps unexpected: they were weaker than expected, especially under the natural challenge scenarios. Furthermore, the difference between the estimates for the natural and artificial challenges was not expected. One mechanism that could affect the estimated correlations is the number of animals culled. However, within reasonable limits (i.e. less than 5% of the total animals removed) this effect is likely to be small. Furthermore, the number of animals removed for the cases for the artificial challenge is greater than the number removed for the low-natural scenario but lower than the number removed for the high-natural scenario. The correlations estimated for the artificial scenario are stronger than those for both the low-natural and the high-natural scenario. This mechanism alone would not explain the differences between artificial and natural scenarios.

A more likely explanation for the difference between the artificial and natural scenario results is that in the artificial scenario the animals receive a fixed, predetermined, larval dose, irrespective of their food intake. Therefore, this difference in determining the level of individual challenge potentially explains the difference of the correlations estimated for the artificial vs. the natural scenario. The impact of this difference may be illustrated with an example. Imagine two lambs at the start of the grazing period, animal 1 with a higher food intake than animal 2, but with the same live-weight and level of resistance. Animal 1 will eat more grass, consuming more infective larvae than animal 2 and therefore will have a greater growth penalty. Subsequently, this would be reversed since animal 2 will grow faster, hence eat more than animal 1 and face a greater larval challenge. This self-correcting effect recurs continuously and could possibly have an impact on the correlations between faecal egg count and production traits.

5.4.3 Interpretation of the results

The results for the correlations obtained from the natural challenge scenarios indicate that there would not be a benefit (in terms of live-weight) of including faecal egg count in a selection index, as they are not significantly different from zero. However, from the means of the different scenarios it is quite apparent that there is a significant benefit from reducing the challenge that the animals face. Viewed from another aspect, the high levels of challenge will have an impact on the mean of production traits but will not necessarily change the ranking of the animals with respect to productivity. As a result, the genetic correlations between faecal egg count and production traits are not sufficient for deciding if resistance should be included in the selection index.

If faecal egg count *per se* is included in the selection objective, the correlations obtained in this study show that at least there will not be any adverse effect on the production traits. Moreover, improving the resistance of the lambs could exploit environmental effects as shown by Bishop and Stear (1999).

An important result emerging from this study is that there is not predicted to be a significant genotype x environment interaction as quantified by the correlations between traits, as far as the dietary protein and challenge level are concerned. The correlations estimated at one level of dietary protein content will be largely valid for other levels of dietary protein contents and levels of challenge.

Another question likely to be asked by a breeder is if the ranking of the animals, in terms of productivity, is likely to change in different environments. Using this model, the phenotypic correlation for the low-natural challenge, within a challenge level, between the 13% and 19% protein levels was estimated to be 0.91 for food intake and 0.94 for live-weight. Similar

results were obtained for different levels of larval challenge and different protein levels. Therefore, the ranking of the animals is virtually the same for the two different and most extreme dietary protein levels (ignoring the 10% dietary protein level). Animals selected on one set of environmental conditions are expected to have the same ranking (in terms of performance) in another set of environmental conditions, these conditions being defined by challenge level and dietary protein content.

A model was developed for examining the genotype × nutrition interaction (with respect to protein), for lambs infected with gastrointestinal nematodes. The model predicts that the level of parasitic challenge and the protein content of the diet will have an effect on the mean of production traits but not a significant impact on the correlation of resistance with production under natural grazing conditions. A genotype x environment interaction of practical significance has not been observed with respect to challenge level and dietary protein content. Therefore, although dietary protein level will influence performance and resistance, it is not predicted to have an influence on the design of breeding programs for nematode resistance.

6. General discussion

In this thesis some aspects of the genetics of resistance to nematodes of small ruminants are explored. There is evidence that genetic variability exists for resistance to gastrointestinal nematodes in small ruminants and especially in sheep. These genetic differences between animals provide an opportunity to exploit this variation in breeding programmes. However, there are still gaps in our knowledge, particularly in areas that relate to the design and implementation of breeding programs. For example, there are very few estimates of genetic and phenotypic parameters for resistance to parasites in goats. Even in sheep where many more parameter estimates have been obtained, studies have produced a diversity of genetic estimates (e.g. r_g between faecal egg count and live weight range between: -0.8 to 0.4). Thus there is still need to estimate genetic and phenotypic parameters and especially to understand why specific results have been obtained. It is possible that various management techniques and components of the environment, such as the level of various nutrients, affect the estimates of the genetic and phenotypic parameters.

Two chapters comprise parameter estimation, one using data obtained from goats and analysed using repeatability and multivariate models, the other using a random regression model to analyse data collected on lambs. The two species of small ruminants are infected by the same gastrointestinal parasite species; the documented difference between them is that goats are more susceptible to parasites than sheep (e.g. Le Jambre *et al.* 1976).

Two further chapters cover two aspects of the management of animals that could have an effect on a breeding program that includes resistance to nematodes, and which are examined by means of computer simulation. The first (Chapter four), is the possible exploitation of the particular form of the distribution (and the epidemiology) of faecal egg counts in a population of animals in order to reduce the number of larvae on pasture and, subsequently,

the challenge of the animals and thus improve the response to selection in production traits. The impact of separation/culling combined with long-term selection was also examined. The second aspect dealt with (Chapter five) is the protein intake of the animals, which is assumed to affect the ability of animals to mount resistance against parasites, and the impact it has on the estimates of genetic and phenotypic parameters.

In chapter two data collected from goats were analysed. Resistance to gastrointestinal parasites was found to be a heritable trait of kids, albeit to a lower degree compared to lambs. The correlations between resistance and production traits, both genetic and phenotypic, were found to be close to zero. The heritability of the production traits was found to be moderate to high.

There have been very few studies estimating genetic parameters for goats. This is probably due to the fact that sheep production is far more economically important in the developed countries than goat production. The continuation of the study of goat populations will depend on the importance of this species in animal production. Currently the cashmere market is in decline and thus there is not much prospect of funding for the continuation of relevant studies. For example, the bulk of the cashmere used in garment production in Scotland is imported from China. Thus the continuation of research in the resistance of goats in gastrointestinal parasites is not probable in the UK, in the long term.

In other countries like France, Italy, Spain and Greece, with large goat populations, there is a case for research in this area, particularly when goats are grazing and not browsing. This is especially the case when sheep and goats are grazing the same pastures. In these countries the genetic aspects of goats resistance to gastrointestinal parasites should be further explored because: a) goats can function as multiplying reservoirs and can increase the larva load of a

pasture while grazing, b) goats are treated more frequently with anthelminthics compared to sheep and thus the opportunity for parasites to develop resistance to anthelminthics is greater in goats (Kettle *et al.* 1983), c) reduced growth rate due to parasitism is probably greater in goats compared with sheep and thus higher levels of production are lost and d) they might be used as a model to broaden our knowledge of the way in which parasites interact with animals. A future area of research would be to undertake studies that seek to model and quantify the epidemiology of the disease when goats are grazing a pasture, the reduction in their growth rate and the development of anthelminthic resistance of parasites harboured by goats.

In chapter three random regression techniques were applied to data collected from sheep. Random regression techniques allow an interpolation of genetic and phenotypic parameters for data points for which there are no measurements. The application of such techniques understand better the genetic properties the population has and how they change as a function of time. This is very useful in data like faecal egg count, which are very variable, and the environment effects are large. Random regression type models resulted in the estimation of smoother changes over time in the variance components of resistance to gastrointestinal parasite, which is what was expected.

However, as random regression type models are still being developed and improved, there can be problems with their application. An attempt was made to fit a bivariate model for jointly analysing the available faecal egg count and the live weight data. Although the data were available and apparently sound, a bivariate random regression type model would not converge. This analysis would have made it possible to compare the results of the 'traditional' multivariate analyses with random regression models.

In the analyses of faecal egg count using the random regression model, it was found that for animals older than approximately three and a half months, faecal egg counts sampled at different dates represented essentially the same trait as judged by the inter-age genetic correlations. This has implications for breeding programs. Data sampled from different farms at different ages, over three and a half months of age, could be pooled together and analysed as one trait. This adds robustness to the breeding program.

Estimation of genetic parameters and thus the ability to make theoretical predictions is not the finishing line for implementing an animal breeding program. In the parasite – animal system there are obviously two organisms involved. Both of them can be affected by management decisions and environmental factors in general.

Separation or culling of a given percentage of the most parasitised animals was the first management action that was examined. There was no significant difference in production when separation/culling was performed and the remaining animals were compared with the control animals, irrespective of the assumed repeatability of faecal egg count. Therefore, the separation or culling of animals will not have a significant effect on productivity, in contrast to the effects predicted for selection. This theoretical prediction remains to be verified by field experiments.

The second simulation model examined the impact of nutrition on the estimated genetic parameters of a population. This is an area where more, well designed, field experiments are needed. Nutritional experiments looking at the interaction of nutrition with parasitism are invariably very small in scale, and far too small for attempting to estimate genetic parameters. Nutritional differences are one of the reasons that are usually speculated to contribute to the variation in genetic and phenotypic parameter estimates. In this study the

model gave an initial answer, that there would be little effect attributable to different pasture protein levels on the genetic parameters. These results need to be verified under field conditions.

The supplementation of lambs with protein rich feed might be attractive but there are economic disadvantages associated with it. Livestock production is quite competitive and, as with every industry, the cost of production must be as small as possible. Protein rich feedstuffs are expensive and thus will increase the cost of production. Furthermore, the sheep and goat industries have traditionally been developed and utilized in places where protein is acquired only from pasture, with relatively low protein levels being adequate. In other words they have been developed to fill niches that are unsuitable for other forms of agriculture. Therefore, protein supplementation is unlikely to be one of the measures applied against parasites, unless other measures prove to be more expensive.

The results of this model show that the parameters estimated when the flock is kept in a poor pasture would be similar to those obtained if the flock grazes a rich pasture or are supplemented. In other words, the parameter estimates are robust with respect to protein content of the food. Thus, in implementing a breeding program, the nutrition of the animals will have little or no impact on the design of the program. There is no need for farmers to feed their sheep at the same protein levels. An important point should be emphasised here: the genetic parameter estimates are independent of the protein content of the food but the mean performance of the animals is not; this depends upon the protein level.

Both models described in this thesis have the same basis: the model described by Bishop and Stear (1999). The core mechanism describing the infection of host by parasites was essentially the same in both models, however the models substantially differed in many

respects. The first extension is the separation/culling for which the model of Bishop and Stear (1999) was re-written and extended to include the culling/separation procedure. On the other hand, in the nutritional model, parameters such as food intake and live-weight were estimated on a daily basis and there was a continual feedback of these parameters. Therefore, the mechanism with which the nutritional model works is probably closer to reality than the culling/separation model. The nutrition model could also be extending by explicitly modeling anorexia, which is one of the consequences of parasitism.

As stated before, selection for resistance on its own is not the answer to gastrointestinal parasites. There is a need to explore the effect of combining genetic selection with other measures. Most of the novel approaches with a potential to complement anthelminthics have been examined on their own merit. The combined effect of these approaches has not generally been examined. There is a need for assessing the effect of their combination so as to be able to devise proper strategies for dealing with the problem of parasites. Preferably this work should be done by means of well-designed field experiments.

Such studies are under way in New Zealand and Australia and currently in UK, for the identification of major genes. Identification of a gene (or a marker related to a gene for resistance) will result: a) in easier and more accurate selection procedures, and b) selection procedures that will be independent of the level of challenge (Bishop, 2002).

Throughout this thesis it has been indicated that there can be benefits from breeding for resistance to nematodes, but breeding for resistance differs in some respects from other traits. This particularity of disease resistance traits is due to a) the fact that there are two organisms interacting, both of which are affected by the environment and, b) the fact that the performance of one animal affects the performance of other animals. An example where this

particularity is demonstrated is the experiment of Leathwick *et al.* (2002). These authors used sheep derived from selected lines of Perendale sheep in New Zealand, in which the lines were selected for resistance/susceptibility to nematodes. The two lines in this experiment grazed different replicate farmlets, whereas in the previous selection experiment they had grazed the same pastures. On average, over the three year trial, there was an 8-fold difference in ewe faecal egg count and a 13-fold difference in lamb faecal egg count, compared with 4.5-fold differences in lamb faecal egg count when the two lines grazed together. These faecal egg count differences resulted in an average 3.6-fold difference in pasture larval infestation.

These differences in larval challenge and apparent worm burdens also affected their relative performance. Previously, when these lines of sheep were grazed together under equal pasture challenge, the productivity of the susceptible animals was higher than that of the resistant animals. The productivity differences were (in favour of the susceptible line) 1.7kg live-weight, 5% and 8% difference in fleece weight-of lambs and ewes. It should be noted that under New Zealand conditions the correlation between resistance and performance is often unfavourable, i.e. more resistant animals have poorer performance. However, when the two lines were grazed separately, there was little difference in performance (Leathwick *et al.* 2002). In other words, the performance differences had disappeared. This experiment verifies the predictions of Bishop and Stear (1999) and the type of prediction that the current model makes: the epidemiology of the disease is an important factor to be exploited when breeding for disease resistance. Hence, whereas for other traits 'environment' is to a large extent noise, here it is a dynamic aspect that can be utilised.

Relevant to the above discussion is the effect of clean grazing strategies from the producers and the breeders point of view. While clean grazing might have beneficial economic returns

for the farmer producing animals for the meat market, this might not be the case for a breeder. The breeder will be interested in identifying resistant animals for which the animals would have to be exposed an average amount of larvae. Clean grazing will reduce this challenge and the breeder might not be able to identify differences between animals. Animals with enhanced resistance will then be of value to breeders who are unable to fully implement clean grazing systems.

An area of quantitative genetics which could have a big impact on breeding for resistance to nematodes is the usage of major genes. If suitable major genes (or QTL) are identified one would be able to select resistant animals without the need to expose them to the infectious agent, which is currently the case. It is, however, quite unlikely that a gene conferring complete resistance to nematodes will be found given the fact that all the lambs seem to be susceptible to nematodiasis. Simultaneous selection on many QTLs, although beneficial, will be logistically complex.

As has been pointed out, one must devise an integrated approach, combining different disciplines and planning, for dealing with parasitism in the future. The results from research in different disciplines have to be brought together and combined in the most appropriate way so as to have the optimal result. This has to be done urgently as the levels of resistance to anthelminthics are increasing.

In summary, in both in sheep and goats resistance to nematodes is a heritable trait and thus, there is the opportunity to breed for disease resistance. For sheep, two broad periods with respect to the heritability of faecal egg count were identified: a) before three and a half months of age from three and b) half months of age up to at least six months of age. Thus measurements could be taken at any point in the latter period; the older the lambs though the

better, taking into account the increase in heritability with age. It has been found that the genetic and phenotypic parameters of disease resistance are quite robust and they are not affected by factors such as nutrition level. Thus, it seems that the reason for the very different published estimates is not the feed (protein) that the animals consume, but should be sought elsewhere. As a general summary, the results obtained in this thesis give added confidence to breeding schemes for nematode resistance that combine measurements on lambs from different farms and at slightly different ages.

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